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GASTRIC CANCER: MORPHOLOGIC FACTORS IN FIVE-YEAR SURVIVAL AFTER GASTRECTOMY *

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The prognosis in gastric cancer is generally recognized as poor. Surgical resection offers the best hope of eradication. It has been stated that the over-all cures are less than 5 per cent,[1-3] although in select groups subjected to operation the rate is higher.[4-10] Following recovery from resection, any of the following events with respect to the cancer itself may occur: (a) Fatal termination within 5 years from local recurrence or distant metastases by tumor which was not evident at the time of operation; (b) five-year survival, but subsequent reappearance of the tumor; (c) permanent cure, with no residual cancer, overt or occult.

These old observations raise many questions, such as the following: (1) Is it possible to distinguish between group *a* on the one hand and groups *b* and *c* on the other—between those who will succumb quickly to recurrence or metastases and 5-year survivors? In other words is it possible to forecast outcome? Can factors with prognostic significance be found? (2) What is the explanation for the slow rate of growth of some gastric cancers so that more than 5 years are required for the formation of recurrences or metastases of clinical significance from residual microscopic tumor foci? (3) In 5-year survivors what are the relative proportions of truly cured cases to those with delayed reappearance of residual tumor?

Having at our disposal suitable material, we have sought answers to some of the questions. The material consists of 30 cases in which the patients lived 5 years or longer after resection of a gastric cancer. All available clinical records and gross and microscopic material were

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reviewed. In addition to hematoxylin and eosin stains, the chrome hematoxylin-phloxine and silver impregnation (for argentaffine quality) methods of Gomori were used for cytoplasmic details, and a modified Wilder's silver impregnation was employed to study the finer fibrillar material. Special stains for mucus were made as needed.

It was anticipated that some cases might have to be eliminated from the group because they were not malignant tumors or perhaps even not true neoplasms. This was found to be unnecessary. All cases conformed to the standard criteria for malignant tumors; none were borderline. All exhibited neoplasia, anaplasia, and heterotopia, infiltrating through the muscularis mucosae and, with one exception, into or through the true muscularis.

Study of the 30 5-year survivors revealed some microscopic features which were not expected from our previous experience with gastric cancer. Therefore, a special control group was used for specific and detailed comparison. It was composed of 30 cases of carcinoma of the stomach in which the patients had undergone resections similar to those of the 5-year survivors, had survived operation, but had died of local recurrence or metastases within 1 year after operation. (Hereafter these are designated "short-term" survivors.) Many of them had died within 6 months. Thus, the extreme ends of the growth-rate spectrum were under comparison. It was hoped that this would reveal, to a maximum degree, any differences associated with rate of growth and degree of malignancy.

For a few comparisons with the 5-year survivors, the entire clinical group of 576 cases of gastric cancer was used.[11]

Attempts to find valid criteria for estimating prognosis have been made previously. One of the earliest studies was that of Whipple and Raiford [5] who combined gross and microscopic grading systems and found them useful. Broders [12] made a histologic grading of a large series of cases. The 5-year survival rates were 86.2, 58.8, 30.2, and 23.3 per cent, respectively, in grades 1, 2, 3, and 4, so that there was fair correlation with group prognosis. Dochat and Gray [13] attempted to estimate prognosis by a combination of Dukes' macroscopic classification [14] (originally developed for rectal cancer) and Broders' microscopic grading method.[15] They found the combination better than either alone. Eker [16] also used a combination of gross and microscopic grading and found some, although imperfect, correlation between the results of grading and prognosis. Schindler, Steiner, Smith, and Dailey [17] found little of prognostic value in microscopic grading but greater

value in macroscopic typing. In a later study, Schindler [18] carried macroscopic typing further and observed that the tumors in all 5-year survivors were of the noninfiltrating types I and II. Stout [19] expressed the opinion that histologic grading, based on cell differentiation, was of little value in predicting degree of malignancy, rate of growth, or probable success or failure of surgical treatment.

The consensus seems to be that either macroscopic typing or microscopic grading alone are useful, that combined they are better, but that they can be used only for group and not for individual case prognosis because of frequent discrepancies. In all large series of cases a few patients who should be alive are dead, and others with a supposedly bad prognosis are alive. It was hoped that new and more reliable factors might be revealed by the present analysis.

Prognostic Criteria

Information from Clinical Studies

In earlier studies by some of the members of our group [11,20,21] no reliable clinical prognostic criteria were found. On the contrary, some unexpected and disturbing observations were made. For example, it was noted that short duration of symptoms before operation, however important this might be in selected cases, was of no prognostic significance in this group of 30 5-year survivors. In fact, long duration was more common in the 5-year survivors than in the large group as a whole, exceeding 1 year in 50 per cent of the former (nearly 20 per cent had symptoms for 2 years) in contrast to 30 per cent of the latter. Presence or absence of a palpable tumor also was of no prognostic importance; it was present in 9 of the 5-year survivors as compared with 16 of those who survived less than 1 year. Hemoglobin values, hydrochloric acid content of the gastric secretion, and presence or absence of occult blood in the stools likewise had no prognostic significance.

A comparison of the sex ratios in the 5-year and short-term survivors revealed 18 males (60 per cent) and 12 females (40 per cent) in the former, and 26 males (87 per cent) and 4 females (13 per cent) in the latter group. The corresponding figures for all of our gastric cancers, 576 patients, are 69 per cent males to 31 per cent females,[11] a ratio generally unexplained but like that reported by others. The group of 5-year survivors had a higher proportion of females and the short-term survivors more males than did the entire group. This difference in the prognosis in relation to the sexes remains unexplained.

It corresponds to that in some other cancers, notably carcinoma of the lung,[22] and it may be related to a degenerative change in tumor cells, to be described.

INFORMATION FROM GROSS FEATURES

Location and size of the tumors were of no value in estimating prognosis; they were about the same in the long- and short-term survivors. The exception to this statement was that the latter group included several examples of diffuse, infiltrating carcinoma in which the extent and hence the size of the tumor were not determinable grossly. The largest tumor associated with 5-year survival was 10 by 9 by 6.5 cm.; with less than 1-year survival the largest was 15 by 14 by 8 cm. (843 gm.). The average diameter of the tumor was about 6 cm. in each group.

TABLE I

Comparison of the Macroscopic Type of Tumor in Five-Year and Short-Term Survivors

	Macroscopic types			
	I	II	III	IV
Five-year survivors	9	17	4	0
Survival less than 1 year	1	12	7	10

The only significant prognostic gross factor was the macroscopic typing based on the appearance of the excised specimen. The standards used in gross typing were those previously published,[17] so that it is unnecessary to repeat them. They resemble those advocated by Borrmann.[23] Four types were used, as follows: type I, polypoid carcinoma; type II, noninfiltrating carcinomatous ulcer; type III, infiltrating carcinomatous ulcer; type IV, diffuse infiltrating carcinoma. Those cases in which the cancer was well delimited by distinct raised margins had the best prognosis, but the correlation was not perfect.

The results of the macroscopic comparison of the two groups are given in Table I. All persons except one with a type I tumor survived for 5 years and all with type IV tumor were dead within 1 year, but between these extremes lie two-thirds of the cases with overlapping in type and prognosis. In these, typing was of no prognostic aid. No doubt if cases with intermediate length of survival, namely, 2, 3, and 4 years, were tabulated, the correlation with types I and IV would be less striking. Macroscopic typing used alone would have given a bad prognosis to 4 (types III and IV) of the 5-year survivors, and a good

prognosis to 13 (types I and II) of the short-term group, introducing such a large error as to make the method of little prognostic value.

INFORMATION FROM MICROSCOPIC FEATURES

Three microscopic features were found which were correlated with long survival. In the order of their importance they were: Sharp circumscription of the tumor; two special histologic types; and a characteristic retrogressive change in tumor cells. They will be described in that order, and followed by several other microscopic findings of lesser importance.

Circumscribed Growth

Twenty-five tumors in the 5-year survivors grew in a circumscribed manner, exhibiting a distinct demarcation between tumor and host. The margin of the tumor was usually smooth but sometimes it was scalloped. The advancing border of the tumor was sometimes at the true muscularis, sometimes deep in the muscle layers, and sometimes in the serosa, but it was always fairly sharp, and distinct. *The tumor advanced through the gastric wall en bloc.* This feature is illustrated in Figures 1 to 4. With the lowest magnification this demarcation appeared sharp; with higher magnification a slight amount of intermingling and interdigitation of tumor cells and normal cells was observed occasionally. These cancers, contrary to the implications of their designation, had no crab-like extensions or "roots." The circumscription was not due to a fibrous capsule, which in many cases was incomplete, imperfect, or absent (Figs. 7 to 9). Neither was it due to an unusual degree of mutual cohesiveness of the tumor cells (Fig. 15).

In the 30 cases with survival of less than 1 year, a similar degree of circumscription was found only twice; the remainder were noncircumscribed (Figs. 5 and 6).

Histologic Types

Most of the histologic types of gastric cancer were represented in the group of 5-year survivors, as is shown in Table II, and in Figures 10, and 13 to 16. At first glance there seemed to be no relation of histologic type to prognosis. On closer analysis, however, and by comparison with the short-term survivors, two types of cancer (representing 9 cases) were found only in the 5-year group. They carried a favorable prognosis. They are a special type of undifferentiated carcinoma, to be described, and the well known undifferentiated small cell carcinoma (or sarcoma).

Six undifferentiated carcinomas of a special type formed the largest histologic group associated exclusively with good prognoses (Figs. 7, 8, and 14). These tumors were composed of groups and solid cords of medium-sized polyhedral cells lying in a cell-rich stroma, which comprised about one-half of the area. The cells had large, pale, often irregular nuclei and little cytoplasm. The stroma was rich in cells of the types associated with chronic inflammation. For this reason and because of the basophilic nature of the cancer cells themselves, these tumors had a distinctly bluish appearance under the lower magnifications when stained with hematoxylin and eosin. We have referred to them among ourselves as "the blue cell cancers." These tumors had no products of maturation or function, or other morphologic features by which their histogenetic nature could be identified. With special stains they had no argentaffine granules. With the idea that they might be chief cell carcinomas, they were stained with the chrome hematoxylin-phloxine stain, but they contained no cytoplasmic granules. The

TABLE II

Comparison Between Histologic Classification and Macroscopic Type in Five-Year and Short-Term Survivors after Gastrectomy for Cancer

Histologic type	Five-year survivors, macroscopic type					Surviving less than 1 year, macroscopic type				
	I	II	III	IV	Total cases	I	II	III	IV	Total cases
Adenocarcinoma	7	6	1		14		8	3	5	16
Special undifferentiated carcinoma	1	4	1		6					
Undifferentiated small round cell tumor		3			3					
Mucinous carcinoma		1	2		3	1	1		1	3
Mixed type carcinoma	1	2			3					
Infiltrating scirrhous carcinoma		1			1		3	4	4	11
Totals	9	17	4		30	1	12	7	10	30

cytoplasm had a slight diffuse affinity for the phloxine. The best clues regarding their nature were supplied by 2 cases in which growth of this type was mixed with adenocarcinoma and intermediate forms, and by a third case in which there was mixture with colloid carcinoma. Because they appeared dark and undifferentiated they might, *a priori*, erroneously be regarded as highly malignant. All were sharply circumscribed. One had extensive metastases to regional lymph nodes. Five of these tumors were found in men. There were no tumors of this type in the short-term survivors. This carcinoma should be set apart in classifying gastric cancers. In this series of cases it was always associated with a good prognosis, despite the fact that histologically it would be grade 4.

Three cases in the 5-year survival group were classified as round cell

tumors. They might be reticulum cell sarcomas, although it is not certain that they are not highly undifferentiated medullary carcinomas (Fig. 15). They were circumscribed but nonencapsulated, with tumor cells in direct relation to muscle. The tumors were composed of small to medium-sized round to polyhedral cells growing compactly with little stroma. There was no follicle formation. Silver impregnation for reticulum, however, revealed an abundant network of fibrils which at some point or other made contact with nearly every cell. The patients survived for 5 years despite the fact that metastases were demonstrated in lymph nodes in 2 cases. There were no similar tumors among the short-term survivors. Histologically these tumors also would fall into grade 4, yet the prognosis was good.

Adenocarcinomas formed the largest histologic group in the 5-year survivors, with 14 cases. They presented no unusual features except possibly a remarkably slight secretion of mucus (Figs. 9, 12, and 13). In size the glands varied from small to giant, and in some cases they were mixed. The glands were round, cylindrical, or irregular, and some had papillary ingrowths. The amount of stroma varied from slight to considerable. According to the system of histologic grading previously described,[17] based on the degree of cell differentiation and of deviation from normal appearance of the glands, 5 cases were grade 2, 7 were grade 3, and 2 were grade 4. Twelve of the 14 adenocarcinomas were circumscribed and 2 were infiltrative. The short-term survivors had 16 adenocarcinomas, all of which were noncircumscribed. Adenocarcinomas were associated with favorable prognosis only when they were circumscribed, and not always then.

There were three carcinomas with conspicuous secretion of mucus in each group. One infiltrative scirrhous carcinoma and 3 of mixed type completed the series of long-term survivors. The short-term survivors included 11 examples of infiltrative scirrhous carcinoma, some of which were of the linitis plastica type. Some infiltrated widely like linitis plastica but had in addition a local mass.

Retrogressive Changes

The chief retrogressive change was of a distinctive type. It consisted of atrophy and distortion of tumor cells and pyknosis of their nuclei. This change was most conspicuous at the advancing border of the tumor; less commonly it was seen throughout (Figs. 8 to 11). It was sometimes more striking at the advancing end of a gland than at the proximal end of the same gland (Fig. 12). It appeared to be independent of the character of the stroma. This retrogressive change was most impressive in an undifferentiated, noncircumscribed, scirrhous

carcinoma. This slightly ulcerated, flat, antral tumor measured 4.5 by 4.0 cm. From it there was widespread infiltration of the muscle layers and serosa by scirrhous tumor of linitis plastica type. Everywhere these cells showed marked atrophy and pyknosis (Fig. 10), resembling the histologic picture seen in prostatic carcinomas following orchiectomy. This patient was a female. Altogether, the same change was seen in greater or lesser degree in 14 patients, of which 8 were males.

The common types of degenerative change, such as massive necrosis, were less frequent in the long-term group than in the controls. This may be attributed to their slower rate of growth with better blood supply.

Metastases

Metastases were seen in lymph nodes in 6 of the 18 5-year survivors (33 per cent) in which material was available for study. The 6 cases consisted of 2 each of adenocarcinomas and round cell tumor, and one each of special undifferentiated carcinoma and mixed cell carcinoma. The corresponding figures for the short-term group are 17 cases with metastases in a total of 21 examined (81 per cent). Four in each group also showed infiltration into perigastric adipose tissue. Lymph node metastases cannot be used as an index of prognosis in individual cases although there is some difference in group prognosis. Neither should the presence of regional lymph node metastases *per se* obviate attempts at surgical cure.

Miscellaneous Histologic Features

The tumors of the 5-year survivors contained on the average more inflammatory infiltrate in their stroma than did those of the short-term survivors. This was most conspicuous in the special "blue cell" cancers already described. It was not consistent enough to be used in prognosis. The same may be stated with respect to the amount of dense connective tissue stroma and of tumor necrosis which were usually greater in amount in the short-term survivors. The number of mitotic figures was not discernibly related to prognosis, although counts were not made.

Cure Versus Slow Growth as Explanation for Five-Year Survival

The question whether 5-year survival was due to cure or to slow rate of growth of the tumor cannot be answered as yet from this group of cases because 27 of the patients were alive when last traced. Two of those still living have had recurrences later than 5 years after oper-

ation. In the remaining 25 it is impossible to state how many are cancer-free and will never have a recurrence. One of these patients has, in the meantime, had treatment for two cancers of the face. Of the 3 no longer alive, 2 died of recurrent carcinoma at 22 and 6 years, respectively, after their first operation. The third patient died 7 years after operation, probably from a recurrence.

Thus, while it is impossible to state how many are cancer-free and cured, or how many have slow-growing, subclinical cancer residues, it is known that in 4 (and probably in 5) instances 5-year survival was made possible because the rate of tumor growth was very slow. Curiously, perhaps significantly, all 5 cases had adenocarcinomas.

Cause for Slow Rate of Growth

The cause of the slow rate of growth, whether low aggressiveness of the cancer cells, or high resistance of the host, or a balance between the two, remains unknown. Certain features seen in the 5-year survivors and absent to a comparable degree in the short-term survivors may, however, be the morphologic concomitants of the basic causes and worthy of record.

The most striking change was the atrophy and pyknosis of cancer cells previously mentioned and illustrated in several photomicrographs, notably in Figure 10. The remarkable resemblance to regressing cells in prostatic carcinoma following orchiectomy has been mentioned. It appeared to be primary, independent of the condition of the stroma, and not accompanied by inflammatory reaction. In one widely infiltrating scirrhous carcinoma it was seen in many parts of the growth; in circumscribed tumors it was most striking near the margin. This change involved only tumor cells, the adjacent stromal cells remaining normal in appearance, so that it is probably not on a vascular or nonspecific nutritional basis.

The sharp circumscription of the tumors could, theoretically, be attributed either to low aggressiveness of the tumor or to resistance on the part of the host. Regardless of whether it is the cause or effect of slow growth, it is useful in estimating prognosis.

All 5-year survivors had either a sharply circumscribed neoplasm, or severe retrogressive changes in the tumor cells, or both.

Although the group of 5-year survivors contained a higher proportion of females than did the entire group of 576 cases or the short-term survivors, the 14 cases showing the special degenerative change have the same sex ratio as the entire 5-year group. Their state with respect to hormonal balance is, however, unknown.

Histologic Grading of Gastric Cancer

Histologic grading, however useful it may be in other tumors,[24-26] has not been very helpful in estimating prognosis in gastric cancer.[5,12,13,16,17] There are many grade 1 cases with short survival and some grade 4 cases with unexpectedly long life. The grading systems have been based principally on cell differentiation and on the degree of resemblance to, or deviation from, the normal glandular pattern. The failure of that method is explained by the fact that some forms of cancer which must be given a "bad" grade are now known to be associated with a good prognosis.

Table III

Comparison of Histologic Type and Former Microscopic Grade in Five-Year and Short-Term Survivors after Gastrectomy for Cancer

Histologic type	Five-year survivors, microscopic grade					Surviving less than 1 year, microscopic grade				
	1	2	3	4	Total cases	1	2	3	4	Total cases
Adenocarcinoma		5	7	2	14			3	13	16
Special undifferentiated carcinoma				6	6					0
Undifferentiated small round cell tumor				3	3					0
Mixed type carcinoma				3	3					0
Mucinous carcinoma				3	3				3	3
Infiltrating scirrhous carcinoma				1	1				11	11
Total	0	5	7	18	30	0	0	3	27	30

Table III presents a comparison of histologic type and old microscopic grade in the 5-year and short-term survivors. The special undifferentiated carcinomas and the undifferentiated round cell tumors, now known to have a favorable prognosis, must, by the grading system formerly employed, be given the unfavorable grade 4. Since they constitute nearly one-third of the 5-year survivors, a great error is introduced by them alone. The mucinous, mixed type, and many of the adenocarcinomas were also grade 4, yet their proved course is now known to have been favorable. Altogether, 25 of the 30 5-year survivors were given the unfavorable grades 3 and 4. The grading system was too simple to be successful, and it must now be modified.

Histologic grading appears to work best in tumors derived from simple epithelia in which cells of only one basic type are present. Good examples are the tumors arising from stratified squamous epithelium, transitional epithelium, or simple columnar epithelium, as in the rectum. When more complex epithelia are concerned, such as those in the mammary gland, lungs, and stomach (where the surface mucus-

secreting cells, neck cells, chief cells, parietal cells, argentaffine cells, and sometimes Paneth cells are present), the morphologic potentialities are so complicated that a simple grading system is inadequate. The potentialities of chief and parietal cells in pure tumor form are not known, to our knowledge. It is not known whether they would grow solidly or have lumina. To be successful, any grading system applied to gastric cancer must take into account the complex nature of the epithelium, and the different types of tumor derivable therefrom.

The histologic grading systems which have been applied to gastric cancer have placed chief emphasis on the degree of perfection of the glands, which is a reflection of the differentiation of the individual cells. Some have taken into account also the extent of the tumor and the method of extension. The former was poorly correlated with long survival because 10 of 18 patients in whom microscopic material was available showed metastases to regional lymph nodes (6 cases), or extensions into the perigastric tissues (4 cases), yet they survived for 5 years. However, we have found that the method of extension is very important in and of itself. When it is combined with the other two factors, namely, the two special histologic types and the special degenerative change, an excellent correlation with 5-year survival is found.

Correlation of Gross Type, Histologic Grade, and Histologic Type

In Table II a comparison is made between histologic type (not grade) of gastric cancer and macroscopic type of growth. Except for the special undifferentiated carcinomas and the undifferentiated small round cell tumors, both of which were of localized types (I and II), there is little correlation. Adenocarcinomas and mucinous carcinomas may be of any gross type from I to IV. Scirrhous carcinomas are usually of the infiltrative types III and IV.

In Table III a comparison is made between histologic type and microscopic grade. The adenocarcinomas were distributed among microscopic grades 2, 3, and 4. All other types were placed in grade 4 because the cells appeared highly undifferentiated. The lack of close correlation between histologic grade and clinical outcome has previously been stressed.

A comparison between microscopic grade and macroscopic type is given in Table IV. The latter shows a better correlation with outcome than does the former. There is some slight degree of correlation between them. That it is not greater is probably due to the imperfections in the microscopic grading system which was used.

Comment

This study revealed some factors associated with 5-year survival after gastrectomy which may be used in predicting prognosis. That the correlation was less than perfect may be attributed to the fact that the cancers in 5-year survivors do not constitute a biologically homogeneous group. Long survival may be due either to cure because of the fortuitous circumstance of complete removal of tumor cells, or to slow growth of residual cancer cells. The latter group is biologically homogeneous with respect to degree of malignancy but the former is not, being composed of two possible subgroups. Some may have been cured because, although highly malignant, the cancer cells were all removed; others were, no doubt, of low malignancy but were cured for the same

Table IV

Comparison Between Microscopic Grade and Macroscopic Type in Five-Year and Short-Term Survivors after Gastrectomy for Cancer

Macroscopic types	Five-year survivors, microscopic grades					Surviving less than 1 year, microscopic grades				
	1	2	3	4	Total	1	2	3	4	Total
Type I		3	4	2	9				1	1
Type II		1	3	13	17			1	11	12
Type III		1		3	4			1	6	7
Type IV								1	9	10
Total	0	5	7	18	30	0	0	3	27	30

reason. Inherently, the cells in some of the cases of true cure may be highly malignant and in others of low malignancy, but precise information as to which cases belong in each group is lacking because of absence of a biologic test, the tumors having been removed. The morphologic equivalents to high or low malignancy cannot be accurately determined by a study of this group. Theoretically, the highly malignant tumors should resemble morphologically those in the group of short-term survivors; the presence of such tumors in the 5-year survivor group would presumably be attributable to the removal of all cancer cells. Thus, possibly an error is introduced into any analysis of this group based on morphology. The cancers which recurred after 5 years form a more uniform biologic group in which the common factor is a demonstrated slow rate of growth. At the present time the 30 cases cannot be subdivided into these two biologic groups because the ultimate course is known in only 5. Some years hence, when accurate subclassification becomes possible, it may be possible to establish still more accurate relationships between morphology and prognosis.

The cancers in the group surviving less than 1 year, unlike the 5-year

survivors, constitute a homogeneous biologic group with respect to rate of growth. Inasmuch as they grew from microscopic residues to produce fatal masses within a period of 1 year, they were uniform in having a rapid rate of growth. This homogeneity had its morphologic equivalents in noncircumscribed growth, absence of two favorable histologic types of tumor from the group, and absence of the special retrogressive change in its fully developed form.

It should be emphasized that study of the margins of a gastric cancer in microscopic sections properly prepared is more precise than is gross inspection. The numerous attempts in the past at macroscopic typing have been consistent in demonstrating considerable merit in the method, which often uses sharpness and elevation of the tumor margin as one criterion. However, the microscopic extent of a lesion is frequently greater than is visible to the unaided eye. Eker [16] has seen microscopic extensions 5 cm. beyond the apparent margin of the tumor, and this observation can be confirmed by many others. For this reason degree of circumscription of a tumor as determined microscopically should be more reliable than gross typing.

From the 5-year survivors and the controls it is possible to determine the amount and cause of the error in prognosis by macroscopic typing, histologic grading, or a combination. Four of the 30 5-year survivors would have been given an unfavorable prognosis by macroscopic typing, being of type III (Table II), and 13 of the short-term group would have been given a good prognosis, inasmuch as they were of types I and II (Table II). Twenty-five of the 5-year survivors would have been given a bad prognosis because they were in microscopic grades 3 and 4 (Table II). Only 4 cases would have been given a good prognosis by a combination of microscopic grades 1 and 2 and macroscopic types I and II (Table IV). The error obviously would have been large by any of these methods. Macroscopic typing, alone, would have been better than the combination.

It may be worthy of note that the method used to find factors which have prognostic significance was different in this than in previous studies. It has been customary to apply predetermined criteria or standards to a series of treated cases and to observe the correlations with length of survival. In the present investigation cases with known end-results were analyzed to discover factors not present in a control group of short-term survivors.

Summary

Thirty cases of gastric cancer in which the patients had survived for 5 or more years after resection were compared with a control group of 30 patients who had died of recurrence within 1 year. Efforts were

made to find factors with prognostic significance, explanations for the slow rate of growth observed occasionally, the relative importance of cure versus delayed reappearance of residual tumor cells as an explanation for 5-year survival, and defects in grading systems for gastric cancer.

Factors associated with 5-year survival were sharp circumscription of the tumor, two special histologic types of cancer, and a characteristic degeneration of tumor cells. These factors were more reliable in predicting outcome than were histologic grading or macroscopic typing or a combination of these methods. While the morphologic concomitants of slow rate of growth have been described, the causes remain unknown.

Five-year survival after gastrectomy for cancer may be due to cure by complete extirpation or to slow rate of recurrence. The relative importance of these two factors cannot be determined until the ultimate outcome is known.

The chief defect in macroscopic typing as a method for estimating prognosis lies in its inability to detect the finer degrees of circumscription of the tumor. The chief defects in histologic grading are the failure to recognize the favorable course of a few special types of undifferentiated cancer, and the importance of circumscription and retrogressive changes in the tumor.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 155

Low-power photomicrographs (× 3) to compare circumscribed growth, irrespective of histologic type, in 5-year survivors (Figs. 1, 2, 3, and 4) with infiltrative growth in short-term survivors (Figs. 5 and 6).

FIG. 1. An undifferentiated cell carcinoma which has penetrated through the gastric wall *en bloc*. Figure 7 is a higher magnification showing the details of this tumor and of the junction of tumor and host.

FIG. 2. An adenocarcinoma with similar deep penetration *en bloc* into muscle.

FIG. 3. A circumscribed colloid carcinoma which is growing into muscle. Figure 16 is a higher magnification of this tumor.

FIG. 4. This is an undifferentiated round cell tumor, similar to that shown in Figure 15, which has penetrated through the true muscle layers in circumscribed but nonencapsulated form.

FIG. 5. This is an undifferentiated adenocarcinoma which is not circumscribed. Tumor is seen in the serosa and the perigastric adipose tissue. It is separated from the main tumor by intact muscularis.

FIG. 6. A noncircumscribed mucinous carcinoma with infiltration into the serosa, by-passing intact muscle.

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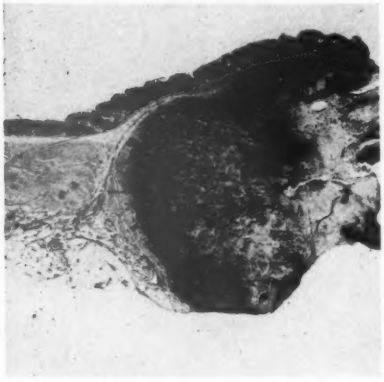

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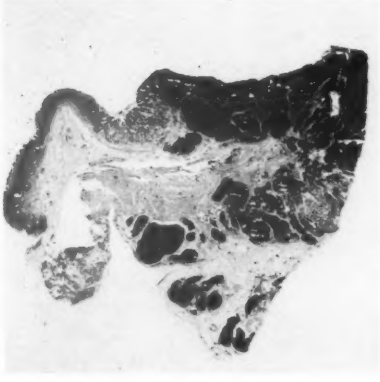
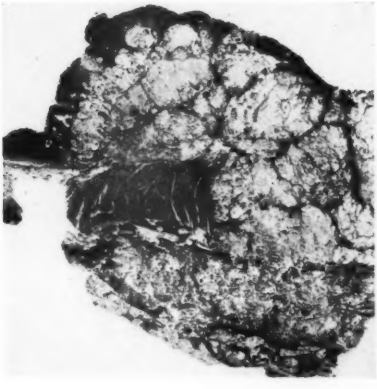




6

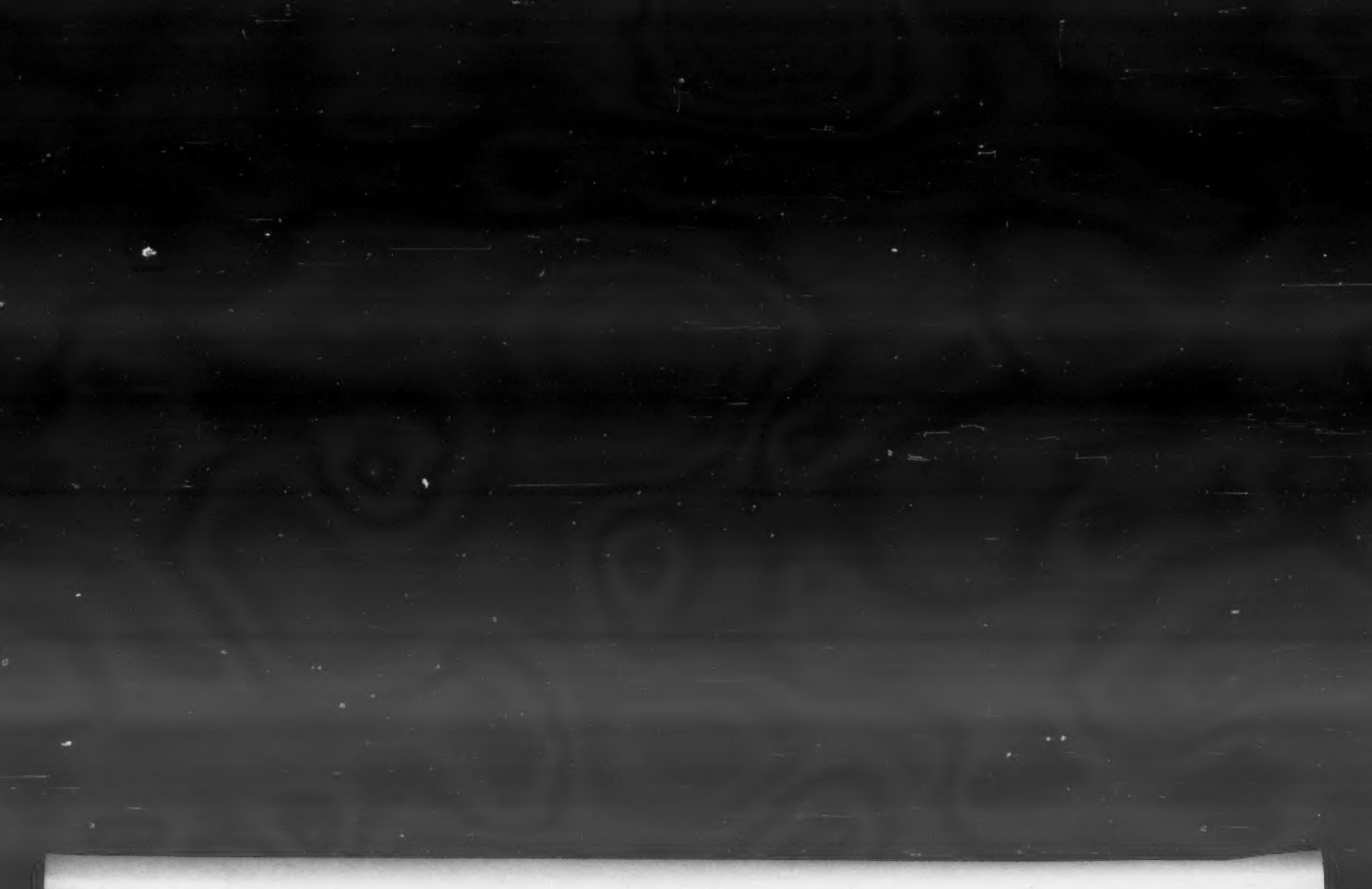
PLATE 156

Three types of histologic reaction (× 200) at the margins of three tumors of two different histologic types.

FIG. 7. Special undifferentiated carcinoma in direct relation to smooth muscle, with no anatomic barrier to explain the circumscribed growth and lack of infiltration.

FIG. 8. Special undifferentiated carcinoma separated from muscle by an intense inflammatory reaction. Many of the advancing carcinoma cells show atrophy and pyknosis. Figure 14 illustrates the center of this tumor.

FIG. 9. Fibrous tissue and inflammatory reaction are interposed between this adenocarcinoma and the adjacent muscle. Some of the outer tumor cells exhibit atrophy and pyknosis. Another view of this same section (Fig. 11) reveals no similar anatomic barrier as an explanation for the circumscription.



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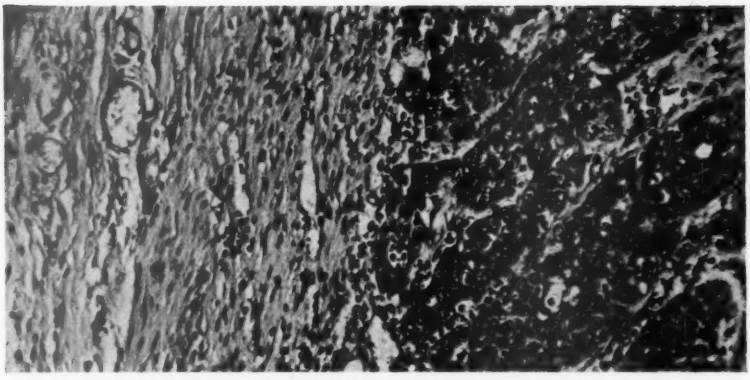

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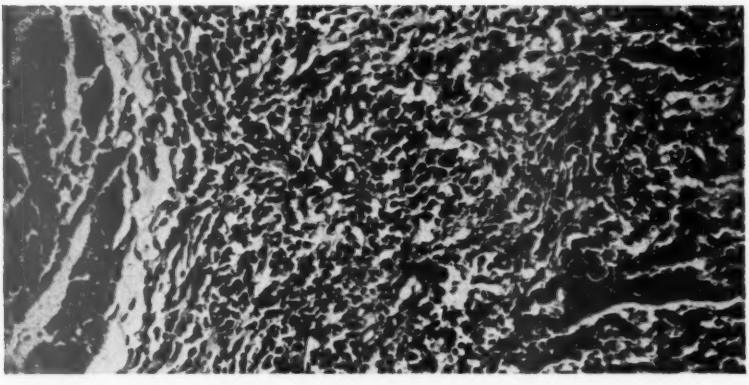

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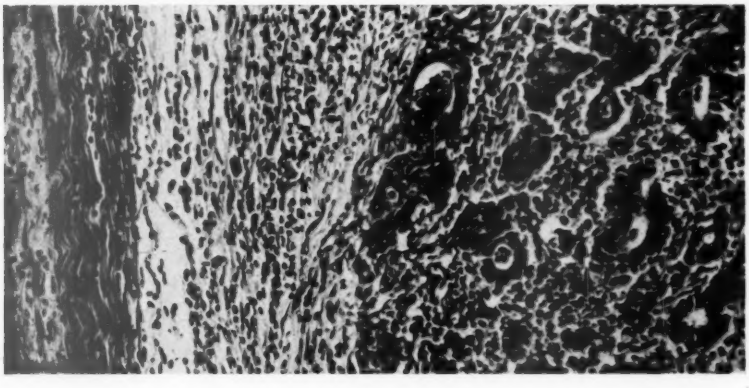

PLATE 157

Three examples of degenerative changes in carcinoma cells as a possible explanation for the slow rate of growth. × 200.

FIG. 10. Widespread infiltrating scirrhous carcinoma showing atrophy and pyknosis resembling that seen in prostatic cancers after orchiectomy.

FIG. 11. Atrophic adenocarcinoma cells mixed with a few lymphocytes are found in edematous connective tissue beneath the muscularis mucosae. There is no anatomic barrier to infiltration by the cancer cells. Same case as shown in Figure 9.

FIG. 12. The adenocarcinoma cells at the margin of the tumor are smaller and darker than those farther back in the same gland.

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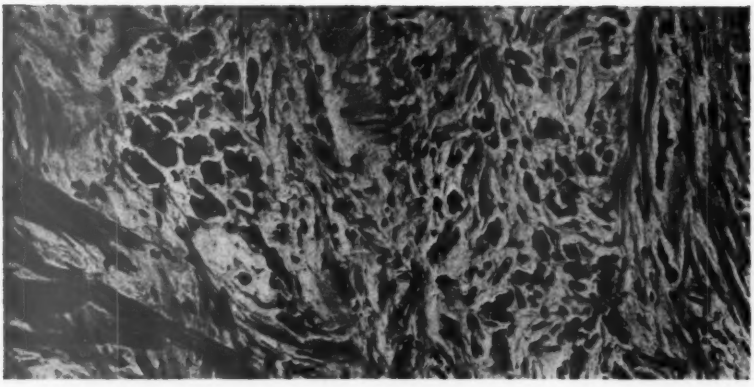

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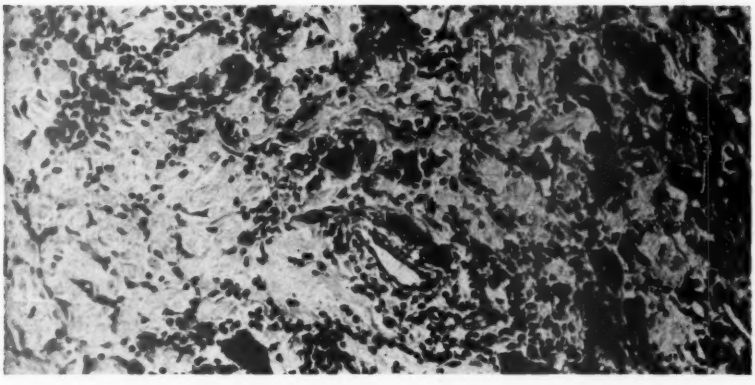

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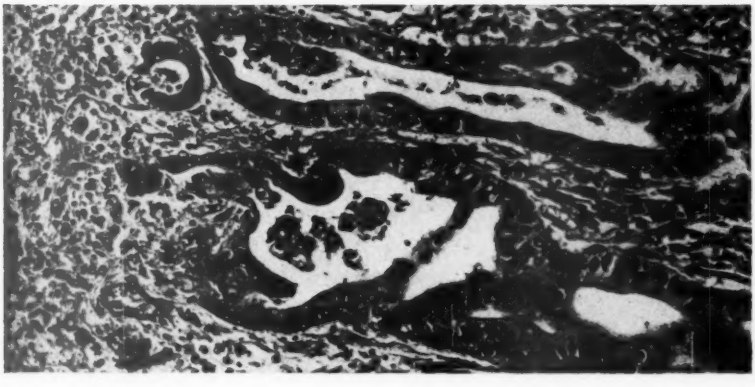

Plate 158

Four of the more common histologic types of gastric cancer are represented in the 5-year survivors (× 200). Two of these types (Figs. 14 and 15) are not present in the series of short-term survivors.

Fig. 13. Metastasis of an adenocarcinoma at necropsy 12 years after gastric resection, and 22 years after excision of a gastric adenoma malignum.

Fig. 14. Special undifferentiated carcinoma of nonscirrhous type. (Another view of this tumor is shown in Fig. 8.)

Fig. 15. Small-celled undifferentiated sarcoma or carcinoma, typical of 3 cases.

Fig. 16. Mucinous carcinoma, typical of 3 cases. (Low-power view is shown in Fig. 3.)

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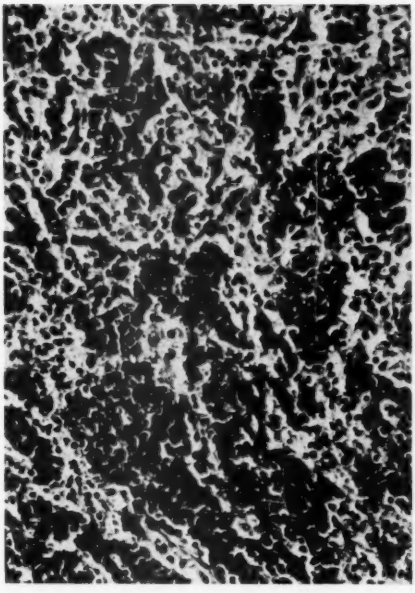

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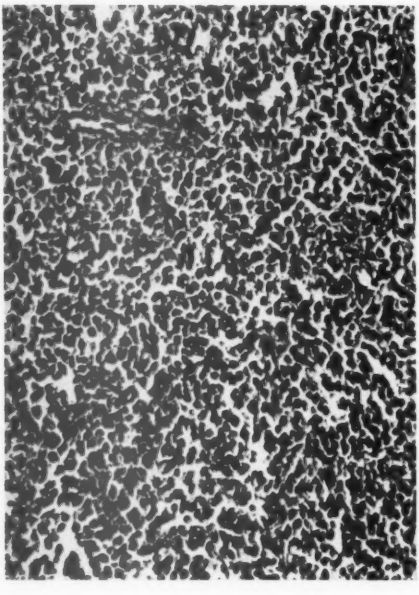

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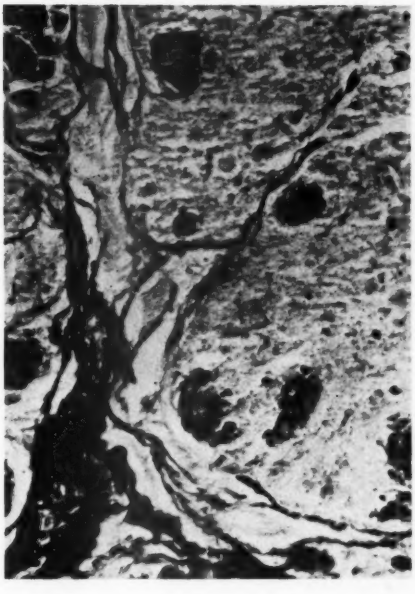

16

HISTOLOGIC FEATURES OF CARCINOMA OF THE CARDIO-ESOPHAGEAL JUNCTION AND CARDIA *

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The greater part of our present knowledge of the growth and behavior of tumors is based on histologic studies. The importance of histologic classification of tumors in determining prognosis and therapy is obvious. The significance of the histogenesis of tumors is not generally so clear. It is possible that a more useful classification of tumors may result from studies directed toward the histogenesis of tumors of similar type in different locations. The advantage of a classification on this basis is exemplified by studies on adnexal carcinoma of the skin.[1-4]

We are presenting a study of the histologic features of a series of carcinomas of the cardio-esophageal junction. This portion of the gastro-intestinal tract offers numerous possibilities for the origin of different types of carcinoma because of the presence of several varieties of epithelium: Stratified squamous epithelium of the esophageal mucosa, mucous epithelium of the free surface of the stomach and the gastric foveolae, the epithelium of the cardiac glands of the esophagus and the stomach, and the epithelium of the ducts and secretory portion of the esophageal glands. The statistics on incidence of carcinoma of the esophagus and of the stomach are not accurate, because of the listing of tumors of the cardio-esophageal portion with either organ. Notkin,[5] in an attempt to determine the relative incidence of carcinoma of the cardia, found that such cases were included indiscriminately with carcinomas of both esophagus and stomach. He suggested isolating tumors of this region in a special group.

LITERATURE ON TYPES OF CARCINOMA OF THE ESOPHAGUS, STOMACH, AND CARDIO-ESOPHAGEAL JUNCTION

Carcinomas confined either to the lowest segment of the esophagus or to the proximal portion of the stomach generally carry the characteristics of the respective organ and are either epidermoid carcinoma or adenocarcinoma, but there are exceptions. Adenocarcinomas sometimes occur in the lower portion of the esophagus, seldom elsewhere.[6-10] Adeno-acanthomas,[11,12] basal cell carcinomas,[8,13] and undifferentiated carcinomas [7,14] of the esophagus also have been reported. Epidermoid carcinomas and adeno-acanthomas do occur in the stomach, but are

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extremely rare. In 1943 Wood [15] reviewed the literature on tumors of the stomach which showed epidermoid characteristics and found 9 examples of pure epidermoid carcinoma and 9 of adeno-acanthoma of the stomach proper. To these he added 2 cases of adeno-acanthoma. In 276 consecutively resected carcinomas of the stomach, we have found 10 cases of adeno-acanthoma, 8 of which occurred at the cardio-esophageal junction and 2 in the pyloric region. We did not find a case of pure epidermoid type.

The carcinomas of the cardio-esophageal junction have not been presented as a special group in the past, but may be found reported with carcinomas of either the esophagus or the stomach. Wood [15] did find reports of 5 cases of epidermoid carcinoma of this junction and one of adeno-acanthoma. An additional case of adeno-acanthoma [16] and 3 cases of epidermoid carcinoma [17-19] are found in the literature. Statements occur frequently in the literature as to the difficulty in determining the origin of tumors occurring at the junction between esophagus and stomach.[19-21] Mallory [19] stated that clinically or by roentgenologic examination it is impossible to distinguish the site of origin, that often it is impossible at autopsy, and that even histologic examination may not settle the issue. For this reason it would appear wise to isolate this group of tumors as Notkin [5] suggested.

General Review of Material

A study has been made of 65 cases of carcinoma of the cardio-esophageal junction collected in the laboratory of the New England Deaconess Hospital from 1927 through part of 1947. These included 59 cases which definitely involved both stomach and esophagus, in addition to 6 cases which occurred so near the junction that involvement could not be ruled out except by serial sections. Fifty-seven of these tumors were resected specimens and 8 were obtained at autopsy. Most of the specimens were fixed *in toto* in Zenker's fixative. Multiple blocks were taken from various areas. All were stained with hematoxylin and eosin and many of them with eosin and methylene blue, van Gieson's stain, Papanicolaou's stain, phosphotungstic acid hematoxylin, Mallory's aniline blue, and Regaud's iron-hematoxylin stain.

A review of the gross aspects of these tumors provided no information for classification in addition to that already recorded for tumors of the esophagus and stomach. It is possible that in the future more detailed descriptions of the gross appearance of tumors in this region may reveal some features which can be correlated with the types isolated on histologic grounds. The chief feature of interest from review

of the gross appearance was the extent of involvement of esophagus or stomach as correlated with the histologic type.

Classification of the tumors on histologic grounds results in four groups: I, Epidermoid carcinoma; II, adeno-acanthoma; III, papillary adenocarcinoma with subgroups A and B; IV, miscellaneous, which includes adenocarcinomas and carcinoma simplex characteristic of carcinomas commonly found in other areas of the stomach. Table I shows the percentage of each group in the total series of carcinomas of the cardio-esophageal junction. Table II shows the extent of involvement of esophagus and stomach as found in the different

TABLE I

Types of Carcinoma at Cardio-Esophageal Junction and Cardia

Type	No. of cases	Percentage
Epidermoid carcinoma	2	3
Adeno-acanthoma (salivary gland type)	8	12
Papillary adenocarcinoma		
Subgroup A	3	5
Subgroup B	16	25
Miscellaneous	36	55
Total	65	

groups. It is evident that each of the groups can involve both esophagus and stomach. It is also to be noted that, relatively speaking, the first three types show a greater tendency to involve more of the esophagus and the fourth more of the stomach.

HISTOLOGY OF THE DIFFERENT GROUPS

Group I. Epidermoid Carcinoma

The epidermoid carcinomas were represented by only 2 cases. There were no unusual features in either, both following the usual pattern of pure epidermoid carcinoma of similar grades (epidermoid I and II) in any other region of the body. Both were resected specimens and both revealed metastases to regional lymph nodes. The metastases presented the same histologic pattern as the primary tumor.

Group II. Adeno-Acanthoma

In group II there were 8 cases. These tumors were tentatively classified as adeno-acanthomas, since that name describes the two most striking histologic patterns in these lesions. However, these particular tumors appeared to be different from adeno-acanthomas in other locations, as will be brought out in later discussion. It is seen in Table II that these tumors may involve esophagus and stomach to approximately the same extent, or the major portion of a tumor may

lie in either organ. The microscopic features were varied. The numerous types of histologic pattern may be summarized as follows.

1. Tumors with various epidermoid characteristics, as definite keratinizing cell masses with typical pearl formation (Fig. 1), single cell keratinization (Fig. 2), cells without keratinization but showing definite intercellular bridges, cells with markedly acidophilic cytoplasm (eosin), cells with large areas of vacuolization in the cytoplasm often containing a flattened pyknotic nucleus (Fig. 3), mosaic pattern of cell arrangement in which cell borders are very sharp and nests of cells with flattened nuclei are arranged in whorl-like formation suggesting early pearl formation (Fig. 2). Special stains aid in emphasizing the different characteristics common to keratinized epithelium,

TABLE II

Extent of Involvement of Esophagus and Stomach by Tumor

Type	Major portion in esophagus	Major portion in stomach	Equal involvement
Epidermoid carcinoma	0	1	1
Adeno-acanthoma (salivary gland type)	2	5	1
Papillary adenocarcinoma			
Subgroup A	1	2	0
Subgroup B	5	11	0
Miscellaneous	0	32	3

e.g., single cells with keratinization stain intense yellow with van Gieson's stain and orange with Papanicolaou's stain; phosphotungstic acid hematoxylin emphasizes intercellular bridges, mosaic pattern, and nests of cells in whorl formation.

2. Papillary adenocarcinoma, in which there are commonly large duct-like structures, usually with a very distinct basement membrane, containing varying amounts of proliferating papillary projections (Fig. 4). In some instances this proliferation is so exuberant and anastomoses and coalescence of papillary projections are so frequent as to form almost solid masses. The epithelium varies from low columnar to very tall cylindrical cells. The former usually have a large round or broad oval nucleus, and the latter a long, narrow, oval nucleus. Either type may show variation in staining, from an intense acidophilic color to an almost clear cytoplasm.

3. Mucinous carcinoma, in which the amount of mucin varies from extensive with large amounts in the stroma and no tumor cells visible to moderate with the glands partially lined by epithelial cells which are markedly distended with mucus.

4. Undifferentiated carcinoma, represented by cell masses of varying

size, in some instances composed entirely of round and polyhedral cells with rather indistinct cell borders and showing a light vesicular cytoplasm and relatively large nucleus. In other instances, similar cells are surrounded by a layer of tall cylindrical cells. Some of these masses appear to have been formed by the coalescence of papillary projections similar to those found in the glands of the papillary adenocarcinoma. There is often honeycombing of such masses by numerous small lumina which at times appear to be spaces left during coalescence of papillae and in other instances to be due to degeneration of a cell or cells to form small cysts.

5. Intimate admixture of different types of cells, with characteristics of keratinizing epithelium, glandular cells and undifferentiated cells (Fig. 5). There was variation in the extent of any one histologic pattern and in the degree of cellular differentiation from case to case. Extremes were represented by one example in which about 50 per cent of the tumor consisted of epidermoid carcinoma, grade I, about 35 per cent adenocarcinoma and undifferentiated carcinoma, and 15 per cent admixture of the three patterns; and by another case showing most of the tumor to be adenocarcinoma and undifferentiated tumor cell masses with honeycombing by numerous small lumina, with only very limited squamous cell differentiation represented by single cells showing early keratinization and vacuolization and by nests of cells in whorl-like arrangement. Four of the 8 cases in this group were autopsy specimens and 4 resected specimens. The 4 autopsy cases showed metastases to the following areas: Regional lymph nodes, pancreas, adrenals, liver, peritoneum, diaphragm, and lung. Two resected cases showed metastases in regional lymph nodes; one case showed no involvement of nodes, and the fourth was not accompanied by nodes. Metastases in most instances showed less differentiation of cells, both as to epidermoid characteristics and glandular differentiation, than was shown in the primary tumors.

Group III. Papillary Adenocarcinomas

There were 19 cases in the group of papillary adenocarcinomas and these were divided into two subgroups. *Subgroup A* consisted of 3 cases which showed portions of the tumor with a pattern of papillary adenocarcinoma, but in which the remainder of the tumor was different from the other 16 cases in this group, different from the usual stomach carcinoma and even different from other examples in this subgroup. *Subgroup B* consisted of 16 cases in which the outstanding histologic feature was that of a papillary adenocarcinoma which grew

into the wall with an inversion of the papillary structure. This inversion of the growth was in contrast to Borrmann's [22] type I, papillary adenocarcinoma of the stomach.

Subgroup A

The three cases, placed together in subgroup A, showed the following histologic pictures in addition to the pattern of papillary adenocarcinoma in each.

Case 1 was an autopsy specimen in which the major portion of the tumor was in the esophagus. The papillary adenocarcinomatous pattern occurred only in the primary tumor. The remainder of the primary tumor and the metastases revealed glands lined by cuboidal cells, anastomosing cords of cuboidal cells, as well as small, solid masses of these cells in which tiny lumina appeared (Fig. 6). The glands were often lined by two layers of these cells. The cells in these areas were fairly uniform in size and shape, containing uniformly small, round, somewhat pyknotic nuclei. The stroma, particularly in the metastases, showed a somewhat myxomatous appearance. Mitotic figures were uncommon. The metastases were found in the liver, skin, subcutaneous tissue, and regional lymph nodes.

Case 2 was a resected specimen in which there was a very small region of papillary adenocarcinoma in the primary lesion. The remainder of the primary tumor and the metastases in the regional nodes showed anastomosing cords of cells, small nests of cells, and cells in adenoid arrangement (Fig. 7). These tumor cells were uniformly small and round with rather acidophilic cytoplasm (eosin) and contained uniformly small, round, centrally placed hyperchromatic nuclei. Mitotic figures were very rare.

Case 3 also was a resected specimen in which the papillary adenocarcinoma occurred in only a small portion of the primary tumor. The major part of the tumor was composed of solid, garland-like, broad strands of cylindric cells. The pattern seen in a part of the area of papillary adenocarcinoma which showed glands lined by tall, cylindric cells suggested that these solid strands of cylindric cells might be formed in some instances by coalescence of papillary projections. There was no tumor in the single node which accompanied the specimen, but carcinoma cells were present in the omental fat.

Subgroup B

Subgroup B was made up of 16 cases which were characterized by an inversion of the papillary structure, expansile type of growth, tendency to cyst formation, and presence of pseudo-stratification and

stratification of epithelial cells. The group was separated from the miscellaneous group on the basis of the following features:

1. Five of the 16 tumors involved largely the esophagus in the gross, as contrasted to the miscellaneous group, in which in not a single instance was the esophagus more involved than the stomach.

2. There was a marked tendency toward large duct-like structures (Fig. 10), often dilated to cystic proportions (Fig. 8), which showed a very distinct connective tissue wall in many instances that separated the tumor into adenoma-like areas (Fig. 9). The growth tended to be expansive rather than infiltrative, as is more common in the usual adenocarcinoma of gastric origin.

3. The tumor tissue of this group tended to involve the submucosal and muscular layers of the stomach rather than the mucosa.

4. The duct-like structures were lined by epithelium that was thrown into varying sized, finger-like branching projections supported by varying amounts of vascular strands of connective tissue. The epithelium sometimes outgrew the connective tissue, forming thick masses or convoluted layers which fused with one another. In such a manner, secondary lumina might honeycomb the more diffuse epithelial masses (Fig. 10). The resemblance of this type of growth to the areas of papillary adenocarcinoma of the adeno-acanthomas in the present series of tumors may be noted. Many times such portions gave the impression of papillomas that were inverted rather than everted and were sometimes referred to as pseudo-papillary tumors (Fig. 9).

5. The lining epithelial cells might be a single cell layer or they might be pseudo-stratified or even stratified in small areas. In either case they might be tall and cylindric or cuboidal, but they tended to be of uniform size (Figs. 11 and 12).

6. The cell outlines were moderately sharp, and there was retention of polarity and basement membrane. Even in the portions with exuberant growth, the cells tended to be more sharply defined than in the more characteristic syncytium-like growth of the usual gastric carcinoma (Figs. 10, 11, and 12).

7. Occasionally a few cells were in whorl-like arrangement, a pattern seen in the adeno-acanthomas.

8. Variable staining reaction of the cytoplasm of the epithelial cells was present, but there was a tendency toward strongly acidophilic staining (eosin). Rarely, this might be so marked in a single cell as to suggest early keratinization.

9. The nuclei were, on the whole, uniform in shape and size, and often lay toward the base of the cells. Most nuclei were long and oval, and many were extremely thin and hyperchromatic, almost pyknotic.

They were relatively small in comparison to the amount of cytoplasm (Figs. 11 and 12). Mitotic figures usually were infrequent, though in some tumors they were more numerous.

10. The cysts usually contained masses of granular, hyalin-like material as well as large numbers of squame-like forms (Fig. 11). The latter apparently represented the desquamation of the long, thin nuclei noted above (stained blue with iron hematoxylin).

11. Necrosis of small cell groups with small cyst formation was common. More extensive necrosis of larger areas of the tumor was not infrequent.

12. Extensive inflammation characterized by large masses of polynuclear cells, lymphocytes, and plasma cells was not infrequent.

13. Mucinous secretion of extensive degree occurred in some tumors. Often there were large cysts lined by tall, cylindric cells distended with mucus. The lumina of the cysts might contain a few cells as well as masses and strands of mucus which often diffused into the surrounding stroma. This type of mucinous secretion was seen in gastric carcinoma, but was somewhat less frequent than is the signet-ring type of carcinoma.

The tumors in this group showed the above characteristics in both the primary growth and the metastases. In the 4 cases on which an autopsy was performed, only one showed distant metastases. In all of the 16 tumors in this group, the glandular differentiation was between 75 and 100 per cent, with 100 per cent in most instances. In some there were small glands like those seen in the usual gastric carcinoma. A sharp line between this group and highly differentiated adenocarcinomas of definite gastric origin could not be drawn, but the above characteristics appeared to be distinct enough to separate cases of that type for further study as to their behavior.

Group IV. Miscellaneous Group

The miscellaneous group of 32 cases included all of the adenocarcinomas that did not fall into group III, subgroup B, as well as the cases of carcinoma simplex and of mucinous carcinoma. It is not necessary to describe in detail all of the variations seen in this group, but merely to point out differences between the glandular tumors of this group and group III, subgroup B. Most of the carcinomas of well differentiated glandular type were of three general types: (1) Papillary form (9 cases), also known as Borrmann's [22] type I or fungating carcinoma, which grew into the lumen of the stomach rather than into the wall, in contrast to those of group III, subgroup B, which we have called papillary adenocarcinoma. (2) Adenocarcinoma of a high degree of glandular differentiation resembling intestinal adenocarcinoma

(5 cases), which were similar to group III, subgroup B, in their sharp delineation of glands with uniform epithelial lining cells and retention of cell polarity and of basement membrane. They nearly always showed a single layer of epithelial cells lining the glands. Duct-like structures and cyst formation were not seen. (3) Adenocarcinomas with varying amounts of glandular differentiation, in which glands were variable in size and, as a rule, were markedly tortuous. Sometimes there was cyst formation in portions of these tumors and then it was often difficult to separate such a tumor from the papillary adenocarcinomas of group III, subgroup B.

In the miscellaneous tumors, there were 18 cases which showed 75 to 100 per cent glandular differentiation. The other cases in this group showed varying amounts of differentiation to glandular form and included a few diffuse infiltrating carcinomas lacking any gland formation.

Embryology and Histology of Cardio-Esophageal Junction

The esophagus, stomach, and respiratory systems all develop from the entodermal layer in the form of a hollow tube. The differentiation of these structures from each other takes place at different stages of development, being completed by the seventh week. At first this tube is lined by a simple layer of low-columnar epithelium. Following the separation of the esophagus, stomach, and respiratory system, the epithelium of the esophagus as well as of the respiratory system is transformed into ciliated epithelium. Then in the esophagus clear, vesicle-like, glycogen-containing elements appear between the ciliated cells. Later these glycogen-containing cells are transformed into superficial squamous cells and partly into ciliated cells. Finally the ciliated cells are reduced in number and are usually completely desquamated at birth, leaving the esophagus lined by stratified squamous epithelium.[23, 24]

The esophagus contains two types of glands, esophageal glands which are found in the submucous layer and superficial glands, termed "cardiac glands," found in the lamina propria mucosae. The esophageal glands are entirely mucus-producing glands.[25] They are formed by epithelial cells growing out from the surface epithelium through the muscularis mucosae in the form of ducts with subsequent glandular development in the submucosa.[26] These structures occur at all levels of the esophagus, vary greatly in number and distribution in different individuals, and often give rise to cysts.[25] The glands are compound with branched tubulo-alveolar terminal portions containing purely mucous epithelium. The epithelium lining the smallest ducts is cuboidal or low-columnar, becoming stratified in the larger ducts, and in the en-

larged main duct the lining is made up of stratified squamous epithelium.[24] These glands are identical with the small mucous glands of the oral and nasal cavities, pharynx, larynx, trachea, and bronchi, some of which are considered to be salivary glands of the mucous type.[27]

The "cardiac glands," given that name because of their resemblance to the cardiac glands of the stomach, are found also at different levels in the esophagus,[23] although they are more prominent at the level of the cricoid cartilage and at the point of transition of esophagus and stomach.[28] The terminal portions have branched and curled tubules containing columnar or cuboidal cells which give the mucin reaction. The excretory ducts are lined by tall-columnar cells similar to the mucous epithelial cells of the gastric foveolae.[24] It is probable that in the descent of the stomach a few rests of cells that are destined to become gastric mucosa remain in the esophageal portion of the gut and become islets of gastric mucosa.[23] At times these glands contain a few parietal cells or zymogenic chief cells which, according to Bensley,[29] indicates, along with other features, that these glands represent decadent or retrogressive structures derived from fundus glands.

The "cardiac glands," as described in the esophagus, extend varying distances into the stomach. In our present study we will confine our interest to this zone. These glands blend gradually with the fundic glands, which contain numerous parietal and zymogenic chief cells. The other types of epithelium of interest in the stomach immediately adjacent to the esophagus are the surface epithelium of tall-columnar, mucus-producing type like that in the ducts of the "cardiac glands" and occasional patches of stratified squamous epithelium which replace the surface epithelium. These patches of squamous epithelium may represent suppression of the gastric glands with replacement of the mucigenous epithelium, as is thought to occur in rodents and ruminants.[29] According to Kirk [30] and Lim,[31] the mucous cells of the surface of the stomach are probably the most primitive of all gastric epithelia, with mucous cells of the cardiac and pyloric glands a little more specialized and with the highest cyto-differentiation shown in the parietal and zymogenic chief cells.

Histogenesis of Certain Tumors of the Cardio-Esophageal Junction

Group I. Epidermoid Carcinomas

The origin of the pure epidermoid carcinomas of the cardio-esophageal junction has been attributed to the squamous cell epithelium of the esophagus.[17] Such tumors could arise in the squamous

epithelial patches in the stomach and extend up to involve the junction. Or they could originate in the glandular portion of stomach mucosa, since such tumors do occur in the stomach at sites too distant from the junction to be attributed to squamous epithelial patches.[15] The histogenesis of the latter type of epidermoid carcinoma is discussed by Wood.[15] Since both of the epidermoid tumors in this series are straightforward keratinizing tumors, the more likely origin is in the esophagus.

Group II. Adeno-Acanthomas

The 8 cases of carcinoma of the cardio-esophageal junction which we have called adeno-acanthomas were given that name on the basis of the combination of glandular cells and cells with characteristics of keratinizing epithelium. We are convinced that the comparison of these tumors with our 2 cases of adeno-acanthoma of the pyloric region of the stomach shows a difference in the histologic pattern and possibly in the mode of origin. We believe the adeno-acanthomas in our group represent tumors to be classified with salivary gland tumors of other regions of the body. These tumors are similar to an adeno-acanthoma of the esophagus which we have reported recently.[11] This case showed certain features which differed from adeno-acanthomas in the stomach (pyloric region),[15] intestine,[32] and gallbladder.[33,34] We expressed the opinion in that report that this tumor probably arose in the esophageal glands, which are mucous glands of the salivary gland type. Reports such as Ahlbom's [35] in 1935 on tumors of the mucous-serous glands of the mouth and Ringertz' [36] in 1938 on similar tumors of these glands in the nasal cavities, accessory sinuses, and nasopharynx emphasized the importance of these glands as a source of carcinoma.

The reader is referred to these authors for comparison of the histologic features and mode of development of these tumors, but, in brief, Ahlbom [35] stated that such tumors show widely varied patterns, sometimes in a single case, *e.g.*, tumors that are mainly adenocarcinoma often show cylindromatous features, papillary cystic structures, or solid epithelial areas resembling basal cell carcinoma or even squamous cell carcinoma; and that squamous epithelial types usually show one or more additional structural types. Often metastases, according to Ahlbom, may differ from most of the primary tumor, though usually the structure occurs in the primary tumor to some extent. Ringertz [36] also described widely varying patterns that are found in salivary gland tumors, even several histologic patterns in one case. It is this tendency to multiplicity of patterns in a single case that is convincing evidence for classification of these 8 cases of combined glandular and squamous cell carcinomas as salivary gland tumors. In the adeno-acanthomas

found in other locations, the pattern is similar to the picture produced by areas of squamous cell metaplasia in normal glandular epithelium. The single cell keratinization, the whorl-like nests of cells, mosaic pattern, and vacuolization of cells are lacking. Of course, that type of adeno-acanthoma could occur also at the cardio-esophageal junction, but we have no cases of this type.

In these 8 cases of carcinoma of salivary gland type of the cardio-esophageal junction and in the case of esophageal adeno-acanthoma mentioned above we have been unable to trace direct origin to any portion of the esophageal glands. Little emphasis has been placed on the submucous glands of the esophagus as a source of carcinoma. Clayton [13] reported an adenocarcinoma of the upper one-third of the esophagus, which he attributed to the esophageal glands. Ahlbom [35] stated that rare reports of basal cell carcinoma of the esophagus may represent tumors of these glands. He referred to a case report of a mixed tumor of the esophagus [37] which he believed might be of the salivary gland type with its origin in the esophageal glands. A rare case report of an adenocarcinoma of the esophagus in regions where the cardiac glands are infrequent suggests that the esophageal glands can give rise to carcinoma.[13, 38] Recently in our laboratory we reviewed a case of resected carcinoma of the esophagus in which the upper portion of the tumor began at the level of the aortic arch and involved 3.5 cm. of the esophageal wall below this level. This tumor showed areas of typical epidermoid carcinoma, grade I, in addition to several carcinomatous glands. In one area there were ducts typical of esophageal gland ducts, which showed early carcinomatous changes. This case is strong support for directing attention to the esophageal glands as a possible source of carcinoma.

Group III. Papillary Adenocarcinoma

Subgroup A

Again in the papillary adenocarcinomas we have tumors which show a combination of patterns. Each tumor revealed a limited area of papillary adenocarcinoma similar to that seen in many of the adeno-acanthomas of group II. In addition, there is an entirely different histologic picture from that of any other tumor of the entire series. The first case, as can be noted from previous description, showed a pattern in the primary growth as well as in the metastases that was very similar to that seen in salivary gland tumors both of the mixed type and of the type seen more frequently in the mouth and nasopharynx, which has been termed basalioma.[35, 36] The second case had a somewhat similar pattern although not as characteristic; but the

marked uniformity of cells as to size, shape, and arrangement, as well as similar uniformity of nuclei, set this apart. Perhaps it represents an undifferentiated carcinoma of the salivary gland type.[36] The third case showed a structural form in the major portion of the tumor that could be compared with Ringertz'[36] solid cylindric cell carcinoma which he found fairly frequently in the nasopharyngeal region. These tumors, he believed, may start as inverted papillary projections of cylindric epithelium in which lumina are formed and then become filled with proliferating tumor cells so that broad, solid strands of cylindric epithelium are formed. Branching of such strands and further coalescence may occur. The epithelium tends to grow in stratified layers and early squamous cell differentiation is not uncommon. However, he stated that the origin of such tumors is not entirely clear-cut, since they might start from surface epithelium of the nasopharynx (columnar epithelium) as well as from ducts of salivary glands, whereas the basalioma type appears to have clear-cut origin in the glandular part of the salivary glands. In line with the possibility of origin from surface epithelium, Kaufmann [39] referred to a solid cylindric cell carcinoma of the stomach described by Hauser, which Kaufmann stated might be classed as the basal cell carcinoma of cylindric epithelial mucosae described by Krompecher. Kaufmann considered such tumors rare, and certainly recent literature does not mention them. It is of interest that the first case in this group showed the major portion of the tumor in the esophagus. It would appear that in the first 2 cases the most likely source was the esophageal glands and that in the third case it could have been in these glands or possibly in the cylindric epithelium of the "cardiac glands" or surface mucosa of the stomach.

Subgroup B

The tumors of subgroup B which have been described as to certain specific characteristics could arise in several different sites: Mucous epithelium of the free surface of the stomach, the gastric pits or necks of gastric glands, the tall-columnar epithelium of the excretory ducts of the "cardiac glands" of the esophagus, ducts of the esophageal glands, or possibly from the terminal portions of any one of the glands. It is not unlikely that different tumors originate in all of these sites, although the pattern does not suggest the last source listed. However, Ringertz [36] pointed out that carcinoma of cylindric cell type and adenocarcinoma supposed to arise in the glandular epithelium cannot be sharply separated. MacCallum [40] mentioned the tendency for tumors of the cervix uteri to develop near the line of transition of stratified into cylindric epithelium. Ewing [41] also referred to such transition

zones in the esophagus as being a predisposing factor for epithelioma. At the cardio-esophageal junction there is ample opportunity for such factors since the transition zones are multiple: Surface squamous epithelium of esophagus to surface mucous epithelium of stomach, and several such areas may appear when there are patches of squamous epithelium in the stomach; squamous epithelium to columnar epithelium of the ducts of the cardiac glands; and squamous epithelium to stratified cuboidal and columnar epithelium in the ducts of the esophageal glands.

Proof of any specific origin for any one tumor of this group is not possible. This is particularly evident when we find similar tumors described as gastric tumors of other areas. Ewing [41] mentioned a tumor of stomach origin, described by Hauser,[42] which he called carcinoma cylindro-cellulare microcysticum which resembled closely some carcinomas of this group. However, this author stated that such tumors are not common and are usually found at the pylorus. On the other hand, Ahlbom [35] and Ringertz [36] in their classifications of types of salivary gland tumors included papillary cystic adenocarcinomas. It is not infrequent to find combinations of papillary cystic adenocarcinoma, alveolar carcinoma, and mucinous carcinoma in a single salivary gland tumor; but such combinations can be found also in carcinomas of gastric origin. Thus the only conclusion can be that we have an adenocarcinoma that tends to be an inverted, somewhat circumscribed papillary tumor, often with cystic degeneration, showing certain specific features in the epithelium, that may arise in the esophageal glands as well as in other tissues. The presence of areas of a very similar type of papillary adenocarcinoma in tumors of group II points to the esophageal glands as a source of the tumors just discussed.

Group IV. Miscellaneous Group

In the miscellaneous group are included all other glandular tumors, as well as the examples of carcinoma simplex and mucinous carcinoma. These tumors are of the same types as those commonly found in the stomach at other sites. The line between those which show a high percentage (75 to 100 per cent) of glandular differentiation and the tumors of group III, subgroup B, cannot be sharply drawn. There will be no attempt to trace specifically the histogenesis of these tumors, since neither the literature nor our study permits any very definite theories. Stout [43] stated that most carcinomas of the stomach are derived from mucous epithelium. Whipple and Raiford [44] indirectly suggested the origin of carcinoma of the stomach in other than the mucous cells. They found that the majority of gastric carcinomas

differ from the carcinomas of other parts of the gastro-intestinal tract, that they are less well differentiated and are therefore more malignant. Such a difference they believed may be due to a greater variety of type and function of epithelial cells of gastric mucosa. Other observers [45] have made similar statements.

It is, however, interesting to record a few points about some of these tumors which may suggest certain theoretic considerations as to specific origin. In this miscellaneous group, 18 cases, or 50 per cent, showed glandular differentiation of 75 to 100 per cent. If we add the 16 cases of group III, subgroup B, also differentiated to this degree, the percentage of those so differentiated is raised to 65.4 per cent of the 52 tumors. In a review of 20 cases of gastric carcinoma of other

TABLE III

Carcinomas with 75 to 100 Per Cent Glandular Differentiation

Type	No. of cases	No. of cases with 75 to 100% glandular differentiation	Percentage with 75 to 100% glandular differentiation
Papillary adenocarcinoma, subgroup B	16	16	100
Miscellaneous group	36	18	50
Total	52	34	65.4

areas, we found only 25 per cent with this degree of differentiation. Schindler, Steiner, Smith, and Dailey [45] in grading 50 cases found only 28 per cent and Dochat and Gray [46] found 33 per cent of 1,045 cases. Borrmann's [22] type I carcinoma is represented in the present series by 9 cases, or 14 per cent (2 cases of squamous cell carcinoma excluded), or 16 per cent if we exclude groups I and II. This is contrasted with 20 cases, or 9.5 per cent, of this type I in 211 cases of carcinoma in other parts of the stomach. In addition, there were 5 cases in the group that resembled adenocarcinoma of the large intestine of a type that often reminds one of a simple adenoma.

Since a high level of glandular differentiation is considered to be an indication that tumors will reproduce more nearly normal tissue, we might expect tumors derived from epithelial cells of simple type and less complicated function to reproduce the normal pattern more frequently. We noted under the review of the embryology and histology of the cardio-esophageal area that the cells of the surface mucous epithelium of the stomach and of the mucous epithelium of the cardiac and pyloric regions are more primitive (and their function probably less specialized) than the cells of the gastric glands of the fundus and antrum. Thus the high percentage of more highly differentiated tumors

in the cardio-esophageal junction may point toward origin in the more simple mucous epithelium, whereas the more anaplastic carcinomas, found more frequently in other areas of the stomach, may represent tumors arising in cells with more highly specialized function.

Behavior of Tumors of the Cardio-Esophageal Junction

Definite conclusions as to the behavior of these tumors cannot be drawn on such a small series. In the future, however, as more tumors are studied from this region, the statistical evidence may be increased to figures which are significant.

In Table IV, the average age and the sex is recorded for each group.

Table IV

Sex and Age Distribution of the Different Types of Carcinoma

Sex	Epidermoid carcinoma	Adeno-acanthoma (salivary gland type)	Papillary adenocarcinoma		Miscellaneous	Total no. cases	Percentage
			Subgroup A	Subgroup B			
Males	1	5	3	15	26	50	77
Females	1	3	0	1	10	15	23
Average age	56 yrs.	62.5	52	55	58.5	56.8	

As is evident, there was no striking difference between any of the groups as to age incidence. The average age in a series of 225 carcinomas of the stomach from other areas, collected in our laboratory, is 56.8 yrs. The age range for carcinoma of the esophagus and stomach is quoted as commonly between 50 and 60 years of age, although either can occur at a much younger or older age level.[6, 47, 48, 49] Ahlbom [35] reported the average age for malignant salivary gland tumors as 52 years, but it may be that those of the type similar to the tumors in the present series would show a different age level.

Males outnumbered the females in each group, and in the entire series there are three times as many males as females. This again is in accord with the reported higher incidence of carcinoma of the esophagus and stomach in the male.[6, 48, 49, 50] Ahlbom [35] found no significant difference in sex incidence of salivary gland tumors. In our group II, which is the group most like the salivary gland tumors, the ratio was 5 males to 3 females; but this group is too small for evaluation.

In Table V is found the percentage of incidence of metastases in the entire series of cardio-esophageal carcinoma, as well as in each group.

The percentage of metastases for carcinoma of the esophagus, which

is not broken down as to type, was quoted by Watson [6] as 71.5 per cent, and by Adams [10] as 65 per cent. For metastasis from gastric carcinoma, Stout [43] found 89.5 per cent of 143 autopsied cases; Whipple and Raiford,[44] 55.7 per cent of 95 resected cases; Dochat and Gray,[46] 59 per cent of 1,045 cases, most of which were autopsied. Our figures on 223 cases of resected gastric carcinomas, excluding the present series, is 64.7 per cent. As a group the cardio-esophageal tumors metastasize to the same degree, 66 per cent, as the gastric carcinomas of other portions of the stomach. It is to be noted, however, that when the series

TABLE V

Frequency of Metastases as to Histologic Type of Carcinoma

Type	No. of cases	Resected specimens	Autopsies		No. with metastases	Percentage with metastases
			Operated	Not operated		
Epidermoid carcinoma	2	2	0	0	2	100
Adeno-acanthoma (salivary gland type)	8	4	0	4	6	75
Papillary adenocarcinoma						
Subgroup A	3	2	0	1	2	66.6
Subgroup B	16	15	3	1	9	56.2
Miscellaneous group	36	34	8	2	24	66.6
Total	65	57	11	8	43	66.1

is broken down into the groups there are some differences which may be significant, even though some groups are very small. Both cases of epidermoid carcinoma showed metastases, 6 of the 8 cases in group II, or 75 per cent, and 9 of the 16 cases in group III, subgroup B, or 56 per cent. As for metastases from salivary gland tumors, it is not easy to compare our findings with previous studies since the location again has something to do with the behavior of the tumor. Ahlbom [35] stated that of the malignant salivary gland tumors, those occurring in regions other than the large salivary glands are more malignant. In general, the group as a whole shows a low percentage of metastases, 25 per cent. But it is to be noted that Ahlbom referred to those salivary gland tumors of undifferentiated type and those showing squamous cell differentiation as the more malignant and stated that these metastasize frequently. This difference in the malignancy of different types of salivary gland tumors was emphasized also by Quattlebaum, Dockerty, and Mayo.[51] Another point of interest is Ahlbom's [35] statement that the salivary gland tumors of papillary-cystic type are relatively benign, although some are more malignant than others and these are often more papillary than cystic.

There are only 11 cases of the resected carcinoma of the cardio-esophageal junction for which we have the final findings. In Table VI the occurrence of metastases is given for these cases at autopsy, as well as for 8 other autopsied cases included in the series.

The incidence of metastases in the 19 autopsied cases is 14, or 73.7 per cent. Of the 6 autopsied cases on which gastric resection had been done, only one showed distant metastases on necropsy. This finding is important in the consideration of treatment of carcinoma in this area. All deaths on the resected cases were due to postoperative complications, but all operations in these cases were performed before 1946.

Comment

Sixty-five cases of carcinoma of the cardio-esophageal junction have been described and analyzed with reference to their possible histogenesis. The information gained from the review and its correlation

Table VI

Occurrence of Metastases at Autopsy

Type of tumor	No. of cases	Operated		Without operation	
		Metastases	No metastases	Metastases	No metastases
Epidermoid carcinoma	0	0	0	0	0
Adeno-acanthoma (salivary gland type)	4	0	0	4	0
Papillary adenocarcinoma					
Subgroup A	1	0	0	1	0
Subgroup B	4	2	1	1	0
Miscellaneous group	10	4	4	2	0
Total	19	6	5	8	0

with similar studies in the literature point to the origin of the adeno-acanthomas and the 2 cases of papillary adenocarcinoma, subgroup A, in the esophageal glands of the esophagus. These glands are to be classed as similar to the salivary glands of the mouth and nasopharyngeal region. There is an additional group of tumors, one case of papillary adenocarcinoma, subgroup A, and all the cases of papillary adenocarcinoma, subgroup B, that may arise in the same type of gland, but the origin of some of these may be in the "cardiac glands" of the esophagus or in the stomach mucosa.

There appears to be some evidence that more well differentiated glandular tumors occur at the cardio-esophageal junction than in other parts of the stomach. It is suggested that this may be due to the presence of gastric epithelium in this area that is of a more simple type. The tumors of papillary adenocarcinoma, subgroup B, show a tendency

to an expansive rather than an infiltrative type of growth with frequent cystic changes which may have some significance in the lower percentage of metastases in these tumors than in any other group.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

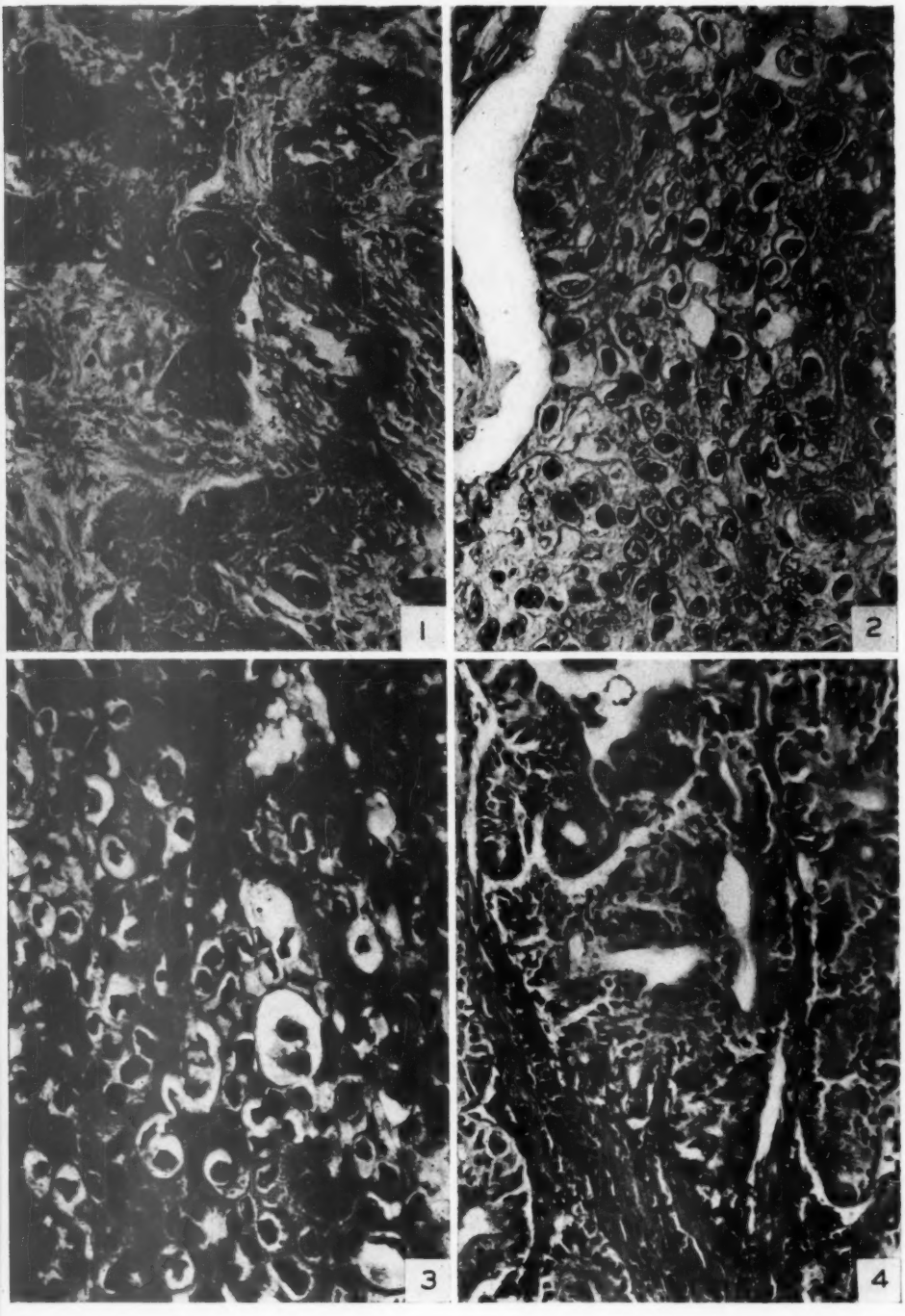
PLATE 159

FIG. 1. Area of keratinizing cell masses with pearl formation. Hematoxylin and eosin stain. × 250.

FIG. 2. Cells in mosaic pattern with keratinization of a few cells. Hematoxylin and eosin stain. × 400.

FIG. 3. Marked vacuolization of cells with flattened pyknotic nuclei. Hematoxylin and eosin stain. × 400.

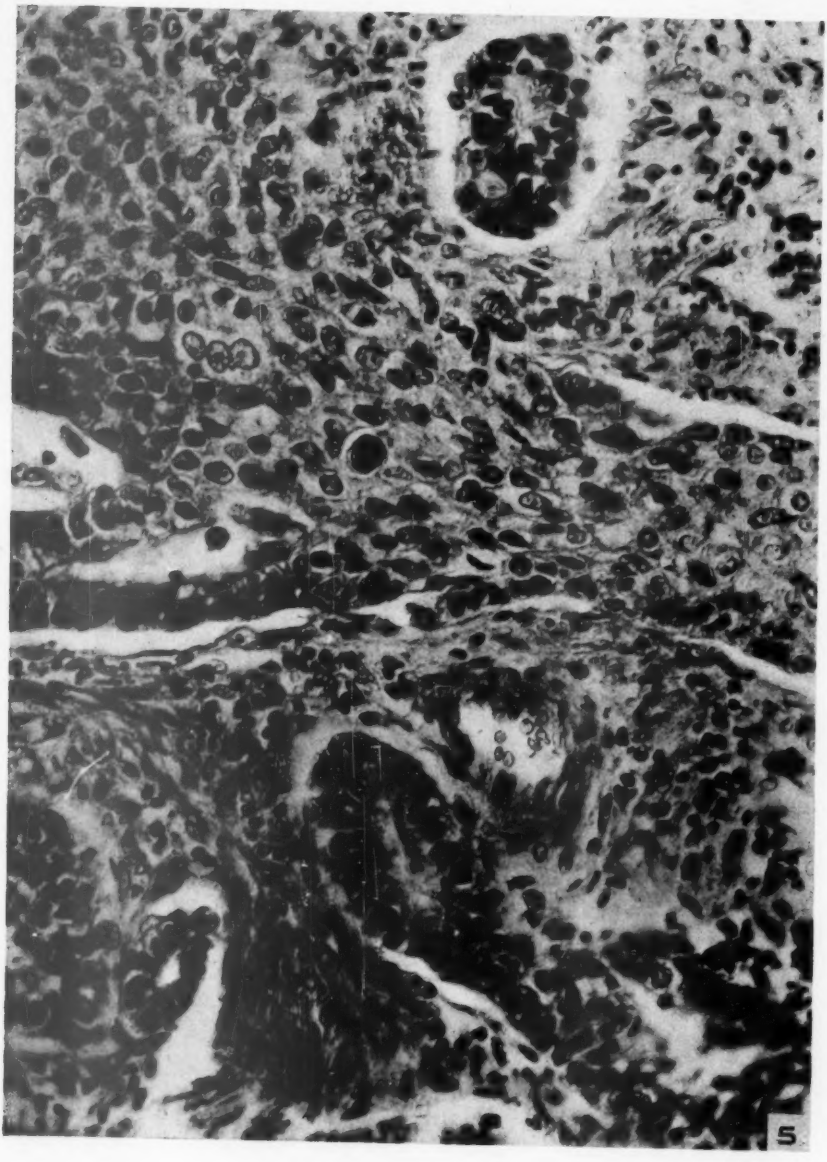
FIG. 4. Papillary adenocarcinoma. Hematoxylin and eosin stain. × 250.



McPeak and Warren Cardio-Esophageal Carcinoma

PLATE 160

FIG. 5. Admixture of undifferentiated cells and glandular cells with single cell keratinization. Papanicolaou stain. × 400.



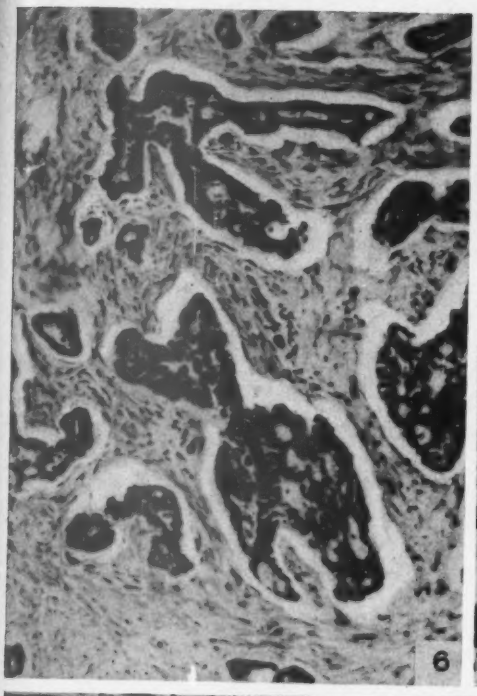
McPeak and Warren Cardio-Esophageal Carcinoma

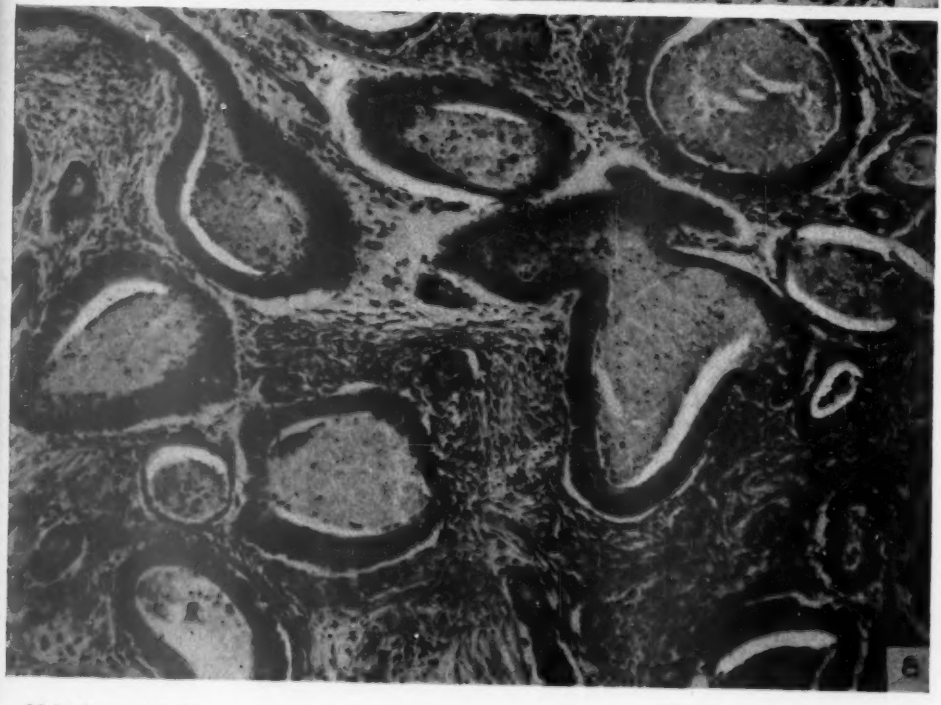
PLATE 161

FIG. 6. Anastomosing cords and masses of cuboidal cells containing lumina in a myxomatous stroma. Case 1, group III, A. Hematoxylin and eosin stain. × 125.

FIG. 7. Anastomosing cords and nests of small cells. Case 2, group III, A. Hematoxylin and eosin stain. × 250.

FIG. 8. Cysts characteristic of group III, B. Hematoxylin and eosin stain. × 125.





McPeak and Warren Cardio-Esophageal Carcinoma

PLATE 162

FIG. 9. Expansile adenoma-like growth of carcinoma. Iron hematoxylin-van Gieson stain. × 35.

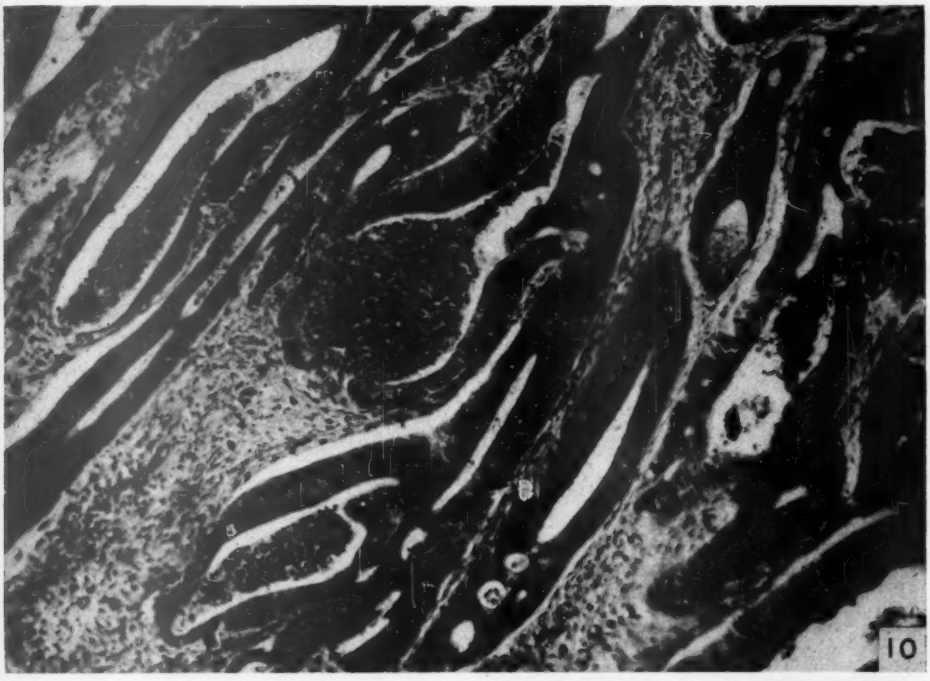
McPeak and Warren Cardio-Esophageal Carcinoma

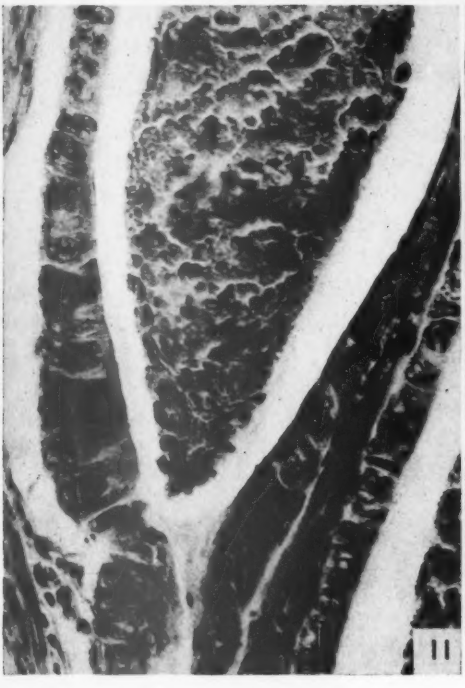
PLATE 163

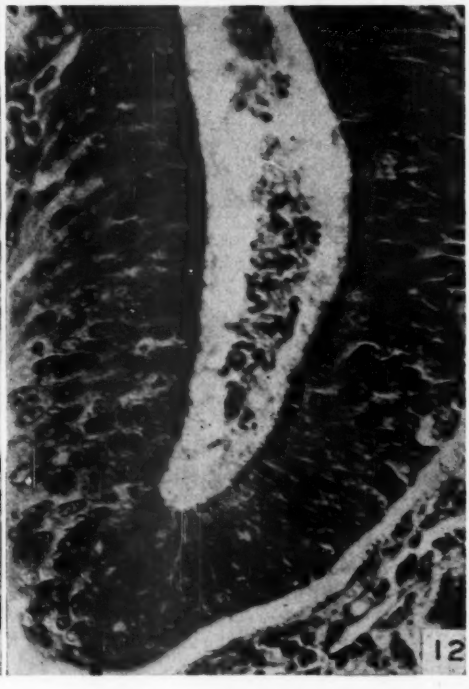
FIG. 10. Duct-like structures in group III, B. Hematoxylin and eosin stain. × 125.

FIG. 11. Single layer of cylindric cells lining duct-like structure. Squame-like material in lumen. Regaud's iron hematoxylin stain. × 400.

FIG. 12. Pseudo-stratification and stratification of cylindric epithelium. Hematoxylin and eosin stain. × 400.







McPeak and Warren Cardio-Esophageal Carcinoma

THE PANCREAS IN UREMIA: A HISTOPATHOLOGIC STUDY *

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In the course of routine examination of histologic sections of the pancreas at necropsy a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of the secretion was observed in a number of cases of uremia (Fig. 1). Because of the similarity of the histologic appearance of the pancreas in these cases to that observed in so-called fibrocystic disease of the pancreas of children,[1-3] it was thought that an investigation of the lesion might be worth while. It was hoped that such a study might give some clues as to the pathogenesis of the similar lesion in fibrocystic disease. With this in mind the following investigation was carried out.

MATERIAL AND METHODS

The histologic appearance of the pancreas was studied in 85 consecutive cases of chronic glomerulonephritis with uremia, in 85 consecutive cases of hypertension (nephrosclerosis) with uremia, and in 100 consecutive cases in which uremia resulted from either hydronephrosis, pyelonephritis, or extrarenal factors. All cases in which the pancreas was the seat of an inflammatory or neoplastic process or in which there was evidence of obstruction of the large pancreatic ducts were excluded from this study. Sections of the pancreas were fixed in formalin, in Zenker's fluid, and in cold acetone. The following stains were used: Hematoxylin and eosin, Congo red, Mallory-Heidenhain's (azocarmine), Mallory's phosphotungstic acid hematoxylin, Weigert's fibrin, mucicarmine, Feulgen's [4] stain for nuclear material, and Gomori's reticulum stain.

The control series consisted of 200 cases in a similar age group in which uremia was not a contributory cause of death.

The lesions were classified as severe, moderate, or mild. If one-half or more of the acini in each lobule of the pancreas were dilated, the lesion was designated as severe; if many, but less than one-half, of the acini in each lobule were dilated, the lesion was considered as moderate; if only a few of the acini in each lobule were dilated, the lesion was classified as mild.

RESULTS

The histologic appearance of the lesion was the same regardless of the cause of the uremia. The lesion, when present, was found in all parts of the pancreas in approximately the same stage of development.

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Sometimes it was slightly more extensive in the tail, but more often it was slightly more pronounced in the head of the gland.

Although the classification did not take into account the extent of the dilatation of the individual acini, it was found that in general the degree of dilatation varied directly with the number of acini involved. In other words, dilatation of the acini was generally greatest in those cases in which the numbers of acini involved were greatest.

The dilated acini were distributed diffusely throughout each lobule and were not concentrated in any particular portion. The severe form of the lesion is represented by Figures 1, 2A and B, and 3A and B. The dilated acini contained an acidophilic stringy material which appeared to be inspissated secretion and occasionally was laminated. Desquamated cells and nuclei occasionally made up a portion of the material, and leukocytes were present rarely. The substance was stained pink by hematoxylin and eosin, blue by the Mallory-Heidenhain stain, tan by the Mallory phosphotungstic acid hematoxylin stain, and blue by the Weigert fibrin stain. Stains for mucin gave negative results.

Associated with dilatation of the acini was a variable degree of flattening of the lining cells. Normally, these cells are pyramidal with the apex toward the lumen. In the dilated acini the cells were cuboidal and occasionally so markedly flattened that one could see only a thin rim of cytoplasm flattened against the basement membrane. The nuclei which normally are spherical were often oval or elongated with the long axis parallel to the basement membrane. The nuclei frequently were pyknotic. Less frequently, karyolysis and karyorrhexis were observed. In addition to the changes in the shape of the cells, there was also a loss of the normal staining reaction. This change could usually be observed best when there was only a mild degree of dilatation. With the hematoxylin and eosin stain the basal portion of the cell, which normally is purple, had an eosinophilic appearance. The zymogen granules in the supranuclear portion of the cell were absent (Fig. 2B). Instead of the two usually distinct zones, the entire cytoplasm had a homogeneous eosinophilic, almost hyaline appearance. From a study of those cases in which the pathologic process was mild and presumably in an early stage of development, it appeared that cytologic changes often, but not always, antedated acinar dilatation.

In some cases in which the degree of dilatation of the acini of the pancreas was severe, there was complete disappearance of a cell or two from the lining. Rarely an entire acinus was destroyed and replaced by fibroblasts and reticulum fibers. This was not a prominent feature in any of the cases, however.

In addition to dilatation of the acini, the tiny ductules were fre-

quently dilated also. Medium-sized and large ducts were not involved in the process, however. Squamation (metaplasia) of the epithelium of the ducts was infrequently observed and, when present, it occurred as an isolated lesion. It was present in 9 cases in which dilatation of the acini was observed and in 4 cases in which there was no dilatation of the acini. In no instance had it led to anatomic obstruction of a duct.

Although vascular lesions, particularly arteriosclerosis, and their sequelae, such as thrombosis, focal infarcts, or regions of atrophy, were frequently observed, they will not be considered in this study except to point out that such lesions were as frequently present in cases in which dilatation of the pancreatic acini did not occur as in cases in which dilatation did occur. Mild to moderate interlobular fibrosis and fatty replacement of the pancreas were frequently observed in both groups of cases, particularly in those subjects more than 60 years of age.

Chronic Glomerulonephritis with Uremia

Examples of the pancreatic lesion in uremia resulting from chronic glomerulonephritis are shown in Figures 1A and 1B. The incidence and the degree of dilatation of the acini in this group of cases are shown in Table I. In the group of 33 cases in which the pancreatic lesion occurred, there were 20 males. The average (mean) age was 33 years, the mean value for blood urea was 344 mg. per 100 cc., and pericarditis was present in 17 cases (52 per cent). Nausea and vomiting were among the symptoms in 24 cases (73 per cent).

TABLE I

Pancreas in Uremia: 85 Cases of Chronic Glomerulonephritis with Uremia

Dilatation of acini of pancreas	Cases	Per cent
Absent	52	61
Present	33	39
Mild	19	58
Moderate	9	27
Severe	5	15

In the group of 52 cases in which the pancreatic lesion did not occur, there were 31 males. The mean age was 34 years, the mean value for blood urea was 253 mg. per 100 cc., and pericarditis occurred in 11 cases (21 per cent). Nausea and vomiting were among the symptoms in 24 cases (46 per cent).

Hypertension (Nephrosclerosis) with Uremia

Examples of the pancreatic lesion in uremia resulting from nephrosclerosis are shown in Figure 2. The incidence and the degree of dilatation of the acini in this group of cases are shown in Table II. Among the 36 cases in which the lesion occurred, there were 25 males. The mean age was 47 years, the mean value for blood urea was 267 mg. per 100 cc., and pericarditis was present in 15 cases (42 per cent).

TABLE II

Pancreas in Uremia: 85 Cases of Hypertension (Nephrosclerosis) with Uremia

Dilatation of acini of pancreas	Cases	Per cent
Absent	49	58
Present	36	42
Mild	22	61
Moderate	12	33
Severe	2	6

Nausea and vomiting were listed among the symptoms in 22 cases (61 per cent).

Among the 49 cases in this group in which the lesion did not occur, there were 40 males. The mean age was 49 years, the mean value for blood urea was 220 mg. per 100 cc., and pericarditis was present in 12 cases (24 per cent). Nausea and vomiting were among the symptoms in 25 cases (51 per cent).

Uremia Resulting from Miscellaneous Causes

Examples of the pancreatic lesion in uremia resulting from hydronephrosis, pyelonephritis, and extrarenal factors are shown in Figures 1C and D, and 3. The incidence and the degree of dilatation of the acini in this group of cases are shown in Table III. Among the 52 cases in which the pancreatic lesion occurred, there were 14 cases of obstructive nephropathy (cases of tubular, pelvic, ureteral, vesical, prostatic, and urethral obstruction), 18 cases of pyelonephritis, and 20 cases in which extrarenal factors were responsible for the uremia. Among these cases there were 39 males and 13 females. The mean age was 58 years, the mean value for blood urea was 224 mg. per 100 cc., and pericarditis was present in 5 cases (10 per cent). Nausea and vomiting were listed among the symptoms in 14 cases (27 per cent).

TABLE III

Pancreas in Uremia: 100 Cases of Uremia Due to Miscellaneous Causes

Dilatation of acini of pancreas	Cases	Per cent
Absent	48	48
Present	52	52
Mild	28	54
Moderate	16	31
Severe	8	15

Among the 48 cases in this group in which the pancreatic lesion did not occur, there were 15 cases of obstructive nephropathy, 15 cases of pyelonephritis, 11 cases of uremia resulting from extrarenal factors, 5 cases of toxic tubular degeneration, and 2 cases of infarcts of the kidneys. There were 32 males and 16 females. The mean age was 49 years, the mean value for blood urea was 227 mg. per 100 cc., and pericarditis was present in 4 cases (8 per cent). Nausea and vomiting were listed among the symptoms in 12 cases (25 per cent).

Control Series

The incidence (20 per cent) and the degree of dilatation of the acini in the control series of 200 cases are shown in Table IV. It should

be emphasized that the incidence of the pancreatic lesion in uremia is significantly greater than in the controls. In none of the cases was a severe degree of dilatation of the acini revealed. The cytologic alterations and the appearance of the inspissated secretion, however, were identical with those observed in the pancreas of uremia. The cases in the control series in which dilatation of the pancreatic acini occurred were analyzed as to the causes of death; the results are shown in Table V. It is probably significant that intestinal obstruction was the most common cause of death in this group. Among the 40 cases in which the lesion occurred, nausea and vomiting were listed among the symptoms in 14 (35 per cent). Pericarditis was absent in all of these cases. Although uremia was not considered a contributory cause of death in any of these cases, azotemia of variable degree had been present in 10 of the 40 cases.

TABLE IV

Pancreas in Uremia: 200 Controls Without Uremia

Dilatation of acini of pancreas	Cases	Per cent
Absent	160	80
Present	40	20
Mild	33	82
Moderate	7	18
Severe	0	0

TABLE V

Pancreas in Uremia: Cause of Death in 40 Controls in Which Dilatation of the Acini of the Pancreas Occurred

Cause of death	Cases
Intestinal obstruction	11
Cancer	7
Infections	7
Congestive heart failure	6
Intracranial lesions	6
Chronic ulcerative colitis	3
Total	40

Among the cases in which the lesion did not occur, pericarditis was present in 7 (4 per cent). Nausea and vomiting were listed among the symptoms in 22 cases (14 per cent).

COMMENT

Anatomic and histologic study of the pancreas gave no clue as to the pathogenesis of these lesions. Although obstruction of the ducts may give rise to the histologic picture described, it is obvious that the obstruction in these cases in which the lesion was generalized would of necessity be at the outlet of the main pancreatic duct. No such obstruction was found and, furthermore, there was no evidence of dilatation of the large and medium-sized ducts. Metaplasia of the ductal epithelium could not be considered as a cause since it was infrequently found both in cases in which the lesion did and in which it did not occur. Furthermore, if metaplasia had produced obstruction, the dilatation of acini would have been localized to the distribution of the involved duct and not generalized. Whether obstruction of acini and ductules by inspissated secretion plays a rôle in pathogenesis remains to be considered.

Interest in the lesion under discussion was aroused because of the similarity of the histologic appearance to that observed in fibrocystic disease of the pancreas. However, the lesions are not identical. In the early stages of fibrocystic disease, dilatation of the acini may not be any greater than that observed in these cases of uremia.[5] However, in the former there is dilatation of the small ducts as well as of the acini, there is always more acinar destruction, and there is decidedly more interstitial proliferation of connective tissue.[5] It may be postulated that if the patients with uremia survived long enough, the histologic appearance of the pancreatic lesion would ultimately be identical with that of fibrocystic disease. Although this possibility must be considered, it cannot be decided with the material available for study.

Wallace and Ashworth [6] observed what appears to be a similar lesion in 45.5 per cent of 200 unselected cases. The changes in the acini described by them occurred as either a focal or a diffuse process, whereas in this study the lesion, when present, was always diffuse. In their study, 56 per cent of patients 50 years of age or over had dilatation of acini, while 38.3 per cent of those below 50 years presented this change. Andrew [7] described locule formation in senile rats and human beings. In photomicrographs, the lesions he described in man do not resemble the acinar dilatation with which this study is concerned. The lesions he described are usually interpreted as foci of atrophy and fat replacement.

It is apparent from the data of the present study that neither age, sex, nor the degree of azotemia played a significant rôle in the pathogenesis of the lesion. From the limited number of cases in which accurate information was available, it appeared that the duration of the uremic state was of no importance in the production of the lesion.

The higher incidence of pericarditis in the cases of glomerulonephritis and nephrosclerosis in which the pancreatic lesion occurred is interesting, but it is difficult to interpret because the factors responsible for uremic pericarditis are not known. It is to be noted also that in the group of cases in which uremia was the result of miscellaneous causes, no such variation in the incidence of pericarditis was found.

It is clear from an examination of the control series that the pancreatic lesion occurs in a variety of conditions other than uremia. The high incidence of intestinal obstruction in the control series suggested that dehydration might be a factor. Dehydration is frequently a complication of renal insufficiency of any type. If dehydration could result in inspissation of pancreatic secretion, it might result in a generalized obstruction of the acini and ductules. In this connection it may be significant that the incidence of vomiting was greater in the cases of

glomerulonephritis and nephrosclerosis in which the pancreatic lesion occurred than in those in which the lesion did not occur. Vomiting would be expected to augment the dehydration in these cases. However, in the group of cases in which uremia resulted from miscellaneous causes there was no significantly greater incidence of vomiting in the cases in which the pancreatic lesion occurred.

In addition to dehydration, other factors which alter the physical and chemical characteristics of pancreatic secretion must be considered. It is a generally accepted fact that nervous (parasympathetic) stimulation of the pancreatic secretion results in the production of a viscid juice rich in enzymes and proteins. After such stimulation the acinar cells shrink and the zymogen granules disappear. Hormonal (secretin) stimulation, on the other hand, produces a more watery juice, poor in enzymes and proteins, without marked histologic changes in the acinar cells. According to Farber,[8] the lesion observed in fibrocystic disease of the pancreas can be reproduced in kittens in all important respects by the injection of parasympatheticomimetic drugs. For this reason, the question arises as to whether the state of uremia is accompanied by excessive parasympathetic stimulation. No satisfactory answer to this question has as yet been obtained. The fact that bradycardia is not a feature of the uremic state suggests that vagus stimulation is not significant.

Another question also arises: Is there an inhibition of hormonal (secretin) stimulation of the pancreas in uremia and intestinal obstruction? Normally, the secretin effect may be elicited by the presence of acids, meat extracts, protein derivatives, fats, fatty acids, soaps, and water in the intestine. If these substances were excluded from the intestinal tract, it is conceivable that the normal release of secretin might not occur and hormonal stimulation of the pancreas might be inhibited. Severe repeated vomiting might bring about such conditions. Repeated vomiting is a feature of both uremia and intestinal obstruction.

If the hormonal (secretin) stimulation of the pancreas were inhibited, the only stimuli to secretion would be nervous (parasympathetic). Such an unbalanced stimulation of the pancreas would result in a viscid juice which, if accompanied by a relative state of dehydration, might become inspissated and obstruct the ductules and acini. Stimulation by secretin has been described as causing a flow of alkaline fluid which serves to flush the alveoli, to thin the juice rich in organic material, and to sweep it along the ducts (Best and Taylor [9]). Because of these considerations, it is suggested that the pancreatic lesion described in this study is the result of an interference with the release and normal

action of secretin brought about by excessive vomiting. If this concept of the pathogenesis of the pancreatic lesion is correct, then one would expect to find it frequently in cases of obstruction of the stomach and small intestine. Such cases are now being investigated.

Does this concept of the pathogenesis of the pancreatic lesion give any clues as to the factors responsible for fibrocystic disease of the pancreas? There is no evidence that excessive parasympathetic stimulation occurs in this disease. Excessive and repeated vomiting likewise is not a feature of this condition. One can, however, postulate that there is a congenital absence of secretin in the intestinal tract or that there is some defect in the mechanism of its release. In support of this viewpoint is the fact that the pancreatic juice is very thick and inspissated and that inspissation of bile and of the secretion of the intestinal glands also occurs (Farber [3]). There is some evidence that, normally, secretin stimulates the secretion of bile and probably the succus entericus (Best and Taylor,[9] Ågren [10]). It has even been called an intestinal diuretic (Ågren). If stimulation by secretin were absent, the obstruction of the small bile ducts and intestinal glands in these cases also might be explained as the result of the production of an abnormally thick inspissated secretion.

It is obvious that more information on secretin is needed. This is particularly true in regard to its presence, mode of formation, and the mechanism of its release in the human being. When this information is available, it may be possible to obtain a better understanding of the pathogenesis of the pancreatic lesion in uremia and of fibrocystic disease of the pancreas.

Summary and Conclusions

Histologic examination of the pancreas at necropsy in subjects in which death had resulted from uremia frequently revealed a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of secretion. The lesion was found in 33 (39 per cent) of 85 cases of uremia resulting from chronic glomerulonephritis, in 36 (42 per cent) of 85 cases of uremia resulting from hypertension (nephrosclerosis), and in 52 (52 per cent) of 100 cases of uremia resulting from miscellaneous causes. Neither age, sex, nor degree or duration of azotemia appeared to play a significant rôle in the production of the lesion. In the control series of 200 cases in which uremia did not occur, the lesion was present in mild or moderate degree in 40 cases (20 per cent). The most common cause of death in the control series was intestinal obstruction. It is suggested that dehydration plus an interference with the release and normal action of secretin

brought about by excessive vomiting are responsible for the lesion. It is also suggested that either a congenital lack of secretin or some defect in the mechanism of its release may be responsible for fibrocystic disease of the pancreas.

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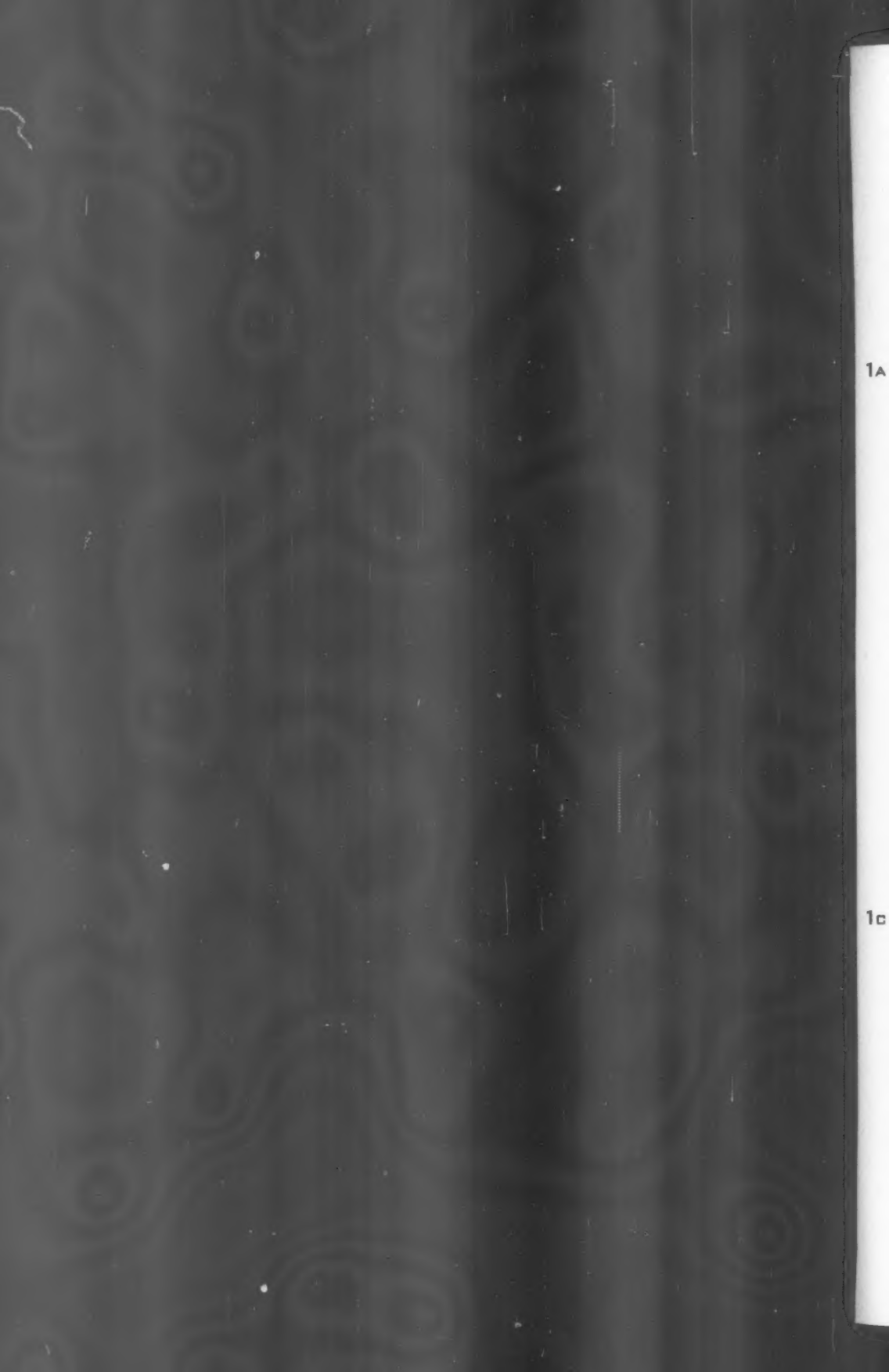
[*Illustrations follow*]

DESCRIPTION OF PLATES

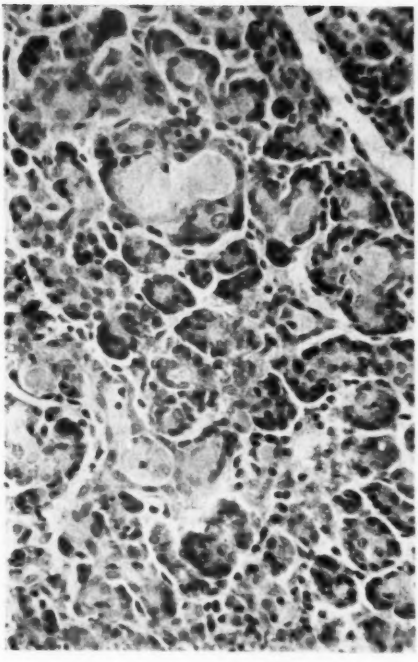
PLATE 164

FIG. 1. Pancreas in uremia. A. In a case of glomerulonephritis; approximately one-half of the acini are dilated. Hematoxylin and eosin stain. × 115. B. Same as A. Hematoxylin and eosin stain. × 275. C. Pancreas in a case of pyelonephritis with uremia, showing dilatation of acini, atrophy of lining epithelium, and inspissated secretion. Hematoxylin and eosin stain. × 115. D. Same as C. Hematoxylin and eosin stain. × 265.


1A
1C

1A

1B



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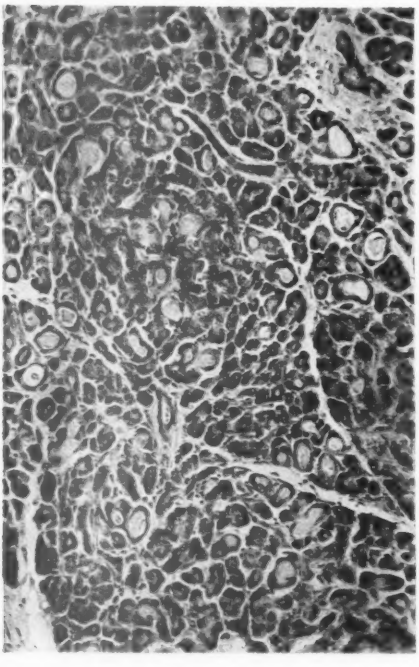

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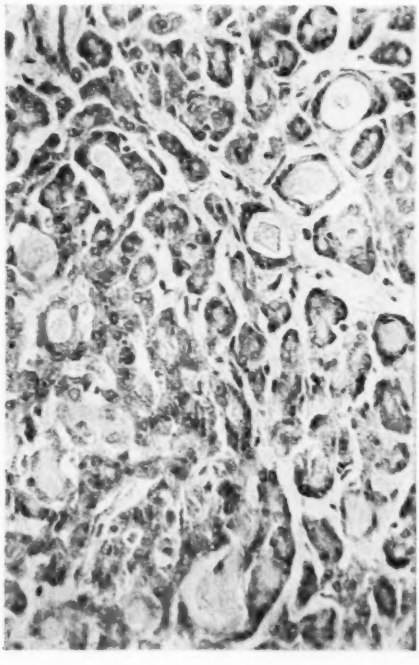
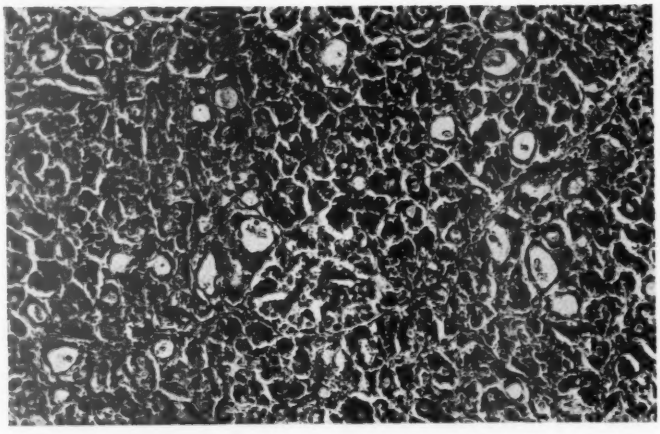


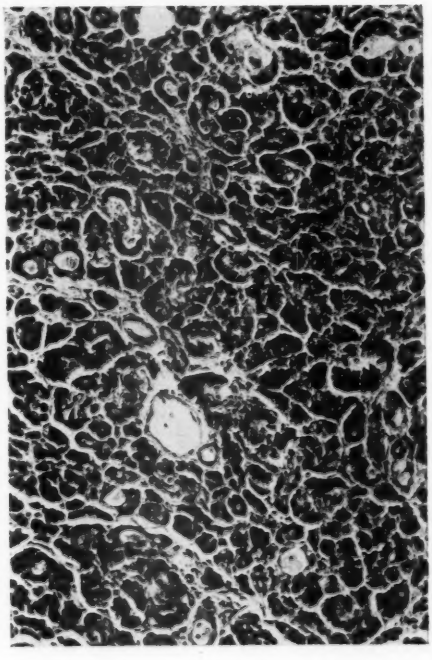
PLATE 165

FIG. 2. Pancreas in uremia associated with hypertension (nephrosclerosis). A. Severe form of the lesion. Hematoxylin and eosin stain. × 115. B. Absence of zymogen granules in epithelial cells of dilated acini. Mallory's aniline blue stain. × 525. C. A moderate number of acini are dilated. Hematoxylin and eosin stain. × 115.

2A



2B

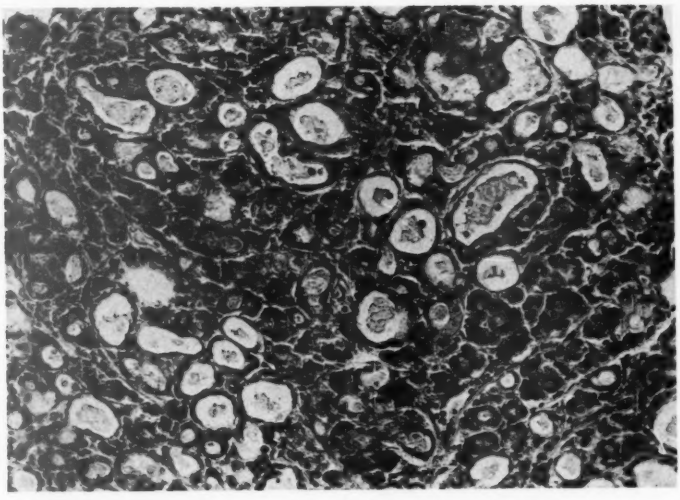


2C

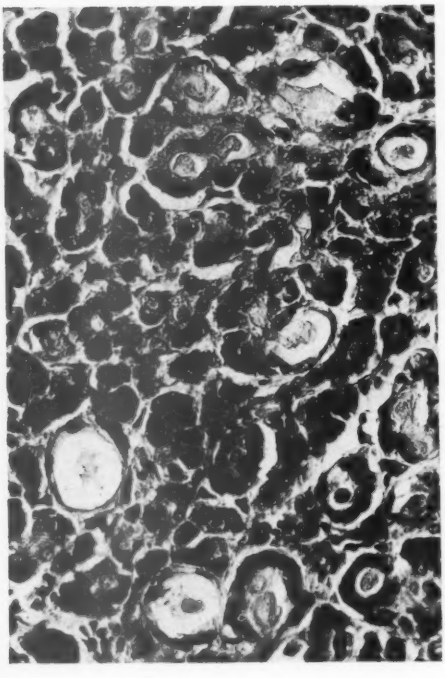
PLATE 166

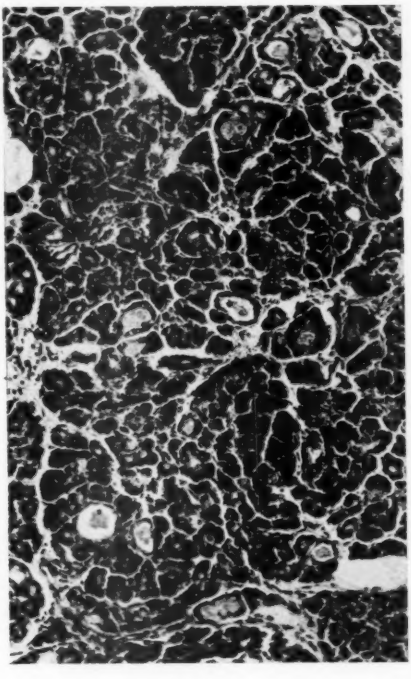
FIG. 3. Pancreas in uremia from miscellaneous causes. A. Pyelonephritis with uremia: a severe lesion. Hematoxylin and eosin stain. × 145. B. Hydronephrosis with uremia; a severe form of the lesion. Hematoxylin and eosin stain. × 265. C. Extrarenal uremia; a moderate number of acini are dilated. Hematoxylin and eosin stain. × 115.

3A



3B





3C

Baggenstoss

The Pancreas in Uremia

SKELETAL EFFECTS OF ESTROGENIC HORMONE IN GROWING VITAMIN C-DEPLETED GUINEA-PIGS *

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The action of estrogenic hormones on the mesenchymal tissues is not yet fully understood. The response varies with species, strain, and sex, and with the dose administered.[1,2] In the growing animal, the main changes consist of hyalinization of connective tissue, vascular walls, and of cartilage associated with increased ossification. This reaction is marked in certain strains of mice and in some birds; it is less pronounced in the growing rat and guinea-pig.[1-5] Inasmuch as some skeletal effects of estrogenic hormone were attributed partly to changes in the connective tissue, it was thought of interest to alter the state of the interstitial substance by depleting animals of vitamin C, and then study the effect of an estrogen. It was hoped, thus, to gain further insight into the mechanism by which the female sex hormone affects the skeleton.

MATERIAL AND METHODS

Twenty-six guinea-pigs weighing 180 to 200 gm. were divided into four groups. *First Series:* Eight animals (normal controls) received a standard diet of purina rabbit chow meal *ad libitum*. This diet is a mixture of checkers with grain, molasses, and chopped alfalfa, and contains the following:

Protein		15.32 per cent
Fat		3.27 " "
Fiber	Carbohydrates	14.88 " "
N free extract	Carbohydrates	53.14 " "
Ash		5.89 " "
Ca		0.84 " "
P		0.32 " "
Carotene		11.0 parts per million
Thiamine		5.09 " " "
Riboflavin		3.74 " " "
Niacin		29.7 " " "

Vitamin C was supplied in the form of fresh lettuce, fed daily. Two animals were sacrificed after 2, 3, 4, and 5 weeks. *Second Series:* Four animals were given lettuce and the standard diet, and were injected subcutaneously with 0.17 mg. of alpha-estradiol benzoate in sesame oil three times weekly.† One animal each was killed after 2, 3,

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4, and 5 weeks. *Third Series:* Six guinea-pigs were fed purina rabbit chow meal without additional lettuce. Two were sacrificed after 2 and 3 weeks; of the remainder, one died during the fourth week; the last had to be killed at the end of the fourth week. *Fourth Series:* Eight guinea-pigs were kept on the vitamin C-free diet and were injected three times weekly with 0.17 mg. of alpha-estradiol benzoate. Two animals were killed after 2, 3 and 4 weeks. Towards the end of the fifth week, the 2 remaining animals appeared very ill and were then sacrificed.

At necropsy, the tibiae, femora, several lower ribs, vertebrae, and the whole heads were removed. The tibiae were measured with

TABLE I

Mean Initial and Final Weights

Experiment	Initial	2 weeks	3 weeks	4 weeks	5 weeks
	gm.	gm.	gm.	gm.	gm.
Normal	189	291	306	317	398
Estrogen	182	255	351	362	371
Avitaminosis C	188	193	191	127	
Avitaminosis C and estrogen	183	196	283	208	212

calipers. The bones were fixed in 10 per cent formalin, and all except the heads decalcified in 5 per cent nitric acid; sections were prepared and stained with hematoxylin and eosin.

GROSS OBSERVATIONS

The mean initial and final weights are presented in Table I. The normal and estrogen-treated guinea-pigs gained weight steadily during the period of observation. The vitamin C-depleted animals failed to gain weight and showed a sudden loss of weight during the fourth week. The vitamin C-depleted animals treated with estrogen gained weight during the first 3 weeks, although less than those receiving alpha-estradiol benzoate and fed the complete diet. During the fourth week, however, they likewise lost considerable weight, although their weight was still much greater than that of the vitamin C-depleted animals not receiving the estrogen.

In animals fed the vitamin C-free diet, periosteal hemorrhages in the long bones and joints, and especially in the lower ribs, appeared after 2 weeks and they became more extensive with increasing duration of the experiment. After 3 weeks, large bead-like swellings at the chondro-osseous junctions of the ribs were noted. The bones were thin and fragile, whereas those of the animals receiving estradiol benzoate were thicker and harder than the normal bones. In the guinea-pigs fed the vitamin C-free diet and treated with the estrogen, rosaries were absent

except for some slight swelling of two ribs in one of 8 animals. Hemorrhages in the periosteal tissues and joints were not seen before the end of the fourth week, and then they were less extensive than in the noninjected vitamin C-depleted animals.

As seen from Table II, the lengths of the tibiae of the normal animals increased steadily in the course of 5 weeks. The tibiae of the guinea-pigs receiving estrogen grew more slowly during the first 4 weeks, but developed a growth spurt during the fifth week. In animals kept on the vitamin C-free diet, an insignificant increase in the length

TABLE II
Mean Lengths of the Tibiae

Experiment	Initial	2 weeks	3 weeks	4 weeks	5 weeks
	cm.	*cm.*	*cm.*	*cm.*	*cm.*
Normal	3.28*	3.45	3.48	3.87	3.93
Estrogen		3.29	3.45	3.49	3.79
Avitaminosis C		3.37	3.61	3.32	
Avitaminosis C and estrogen		3.29	3.46	3.47	3.45

* These values represent means of 4 normal animals weighing 180 gm., and used in former experiments.

of the tibiae was observed during the first 3 weeks; after 4 weeks, the tibiae were shorter than at any earlier period which might be incidental but which might be due also to a collapse of the metaphysis. In vitamin C-depleted guinea-pigs receiving estrogen, the lengths of the tibiae during the first 3 weeks closely paralleled those seen in normally fed animals injected with the hormone. However, a measurable growth spurt occurring in the latter during the fifth week did not take place in the vitamin C-depleted guinea-pigs treated with estrogenic hormone.

HISTOLOGIC EXAMINATION

First Series: Untreated Control Animals

Epiphyses. After 2 or 3 weeks of observation, the epiphyseal plates at the upper end of the tibia were about 350 μ wide and showed a regular configuration (Fig. 1). The cartilage cell rows were separated from each other by thin layers of chondromucoid ground substance. A single column was composed of 10 to 12 proliferating and 4 hypertrophic cartilage cells. After 5 weeks, the growth zone measured 330 μ in height. Moderate amounts of matrix surrounded the individual cell rows which contained 9 columnar and 3 or 4 hypertrophic cells. The three layers of the articular cartilage were distinct, and the ossification of the hypertrophic zone was progressing. The chondro-osseous junction of the ribs and the epiphyseal plates of the femur and vertebrae showed the usual architecture.

Diaphyses. Numerous capillaries eroded the hypertrophic cartilage cells in the metaphysis of long bones, ribs, and vertebrae. In a standard area 1.5 mm. in diameter, 12 to 14 spicules were counted, and after 5 weeks their number had increased to 16 to 18. The trabeculae were slender at the earlier age, but increased in thickness during the 5 weeks of observation. They were covered by a continuous layer of large osteoblasts (Fig. 5). The bone marrow was vascular and cellular. The shaft was composed of medium-sized osteocytes and abundant bony matrix (Fig. 9). The vascular canals were narrow, and the enclosed blood vessels were surrounded by a thin layer of loose connective tissue. The endosteal osteoblasts were numerous and large. The periosteum was thick. Osteoblasts and osteoclasts were present; the latter were situated in grooves of the compacta.

Second Series: Animals Treated with Alpha-Estradiol Benzoate

Epiphyses. After 2 weeks of injections, the growth zones were narrowed to 260 μ. The cartilage cells were smaller and less numerous than in the normal controls, and the matrix was intensely hyalinized and calcified. In the animals receiving 4.99 mg. of the hormone over a period of 3 weeks, a further decrease in the width of the growth zones to 170 μ had occurred (Fig. 2). An individual cell row was composed of 5 or 6 columnar and 3 to 5 small cells of hypertrophic type. The cartilaginous ground substance was heavily calcified, and here and there whole cartilage cell columns had been replaced by thick plugs of sclerosed ground substance (Fig. 6). The articular cartilage cells were smaller than is normal. At the chondro-osseous junction of the ribs and in the femur and vertebrae the growth processes had likewise markedly declined, and regressive changes were accentuated. After 5 weeks, the epiphyseal plate became wider again, the cartilage cells were enlarged, and calcification was less marked.

Diaphyses. After 2 weeks, the metaphyseal capillaries were inconspicuous, poorly filled and accompanied by fibrous tissue. The trabeculae, particularly in their proximal parts, were thicker than ordinarily. This was due chiefly to the presence in their centers of large, unopened cartilage cells still surrounded by some cartilaginous matrix. Along these spicules, a fair number of osteoblasts was noted. The amount of bone present was increased about 25 per cent over normal. The endosteal osteoblasts were delimited from the bone marrow by a layer of fibrillar tissue. The shaft was thick. The haversian canals were narrow and contained poorly filled vessels surrounded by increased amounts of connective tissue. The periosteum was likewise thick and contained much collagen. Near the metaphysis, some osteoclasts were

found, but farther down along the shaft they were scanty or absent. After 3 weeks of injections (Fig. 6), the metaphyseal spicules still contained preserved cartilage cells; they were thick and showed some transverse linking. The peritrabecular connective tissue had increased in amount and the osteoblasts were smaller than is usual. In the distal metaphysis the trabeculae were considerably coarser than in the normal control animal. Conditions in the shaft (Fig. 10) were similar: The vascularization was further decreased, and the bone was solid. Periosteal ossification was advanced, osteoclasts were rare; the shaft had a smooth surface. The periosteum was closely attached to the compact bone, it was dense and contained much collagen; between the fibers, few fibrocytes were seen. After 4 or 5 weeks, the vascularization of the shaft and metaphysis had increased. Numerous large osteoblasts reappeared; the connective tissue was looser than at the earlier experimental stages. Owing to increased resorption of bone, a fibro-osseous network was formed. The vessels perforating the shaft were engorged, the periosteum was looser and contained again more osteoclasts than earlier.

Third Series: Vitamin C-Depleted Animals

Epiphyses. In guinea-pigs kept on the vitamin C-deficient diet for 2 weeks, the growth zones showed the usual width and structure. The proliferation of the columnar cartilage cells and their conversion into hypertrophic cells were not appreciably affected. There may have been a slight decrease in these processes. The cartilaginous ground substance, however, was loose and swollen. After 3 weeks and later, the epiphyseal plate at the upper end of the tibia measured about 325 μ in height and showed an irregular pattern (Fig. 3) owing to the marked swelling of the matrix. The number of cells in an individual column was not changed materially; however, the columnar cartilage cells failed to undergo hypertrophy. Instead of the usual calcium deposits, there appeared in the most distal layer of the cartilage patches of deeply eosinophilic material bearing a certain resemblance to the fibrinoid substance found in the metaphysis. Here and there, capillaries of the bone marrow began to invade the softened epiphyseal cartilage, but many cell capsules were left unopened. The cartilage cells of the joints were decreased in number and size, and there was likewise pronounced swelling of the intercellular matrix. Calcification and ossification of the cartilage were strikingly decreased. The findings at the growth zones of the other bones were similar to those in the tibia. At the chondro-osseous junction of the ribs, a mass of hypertrophic, swollen, vacuolated and poorly calcified cartilage was found.

Diaphyses. After 2 weeks, the metaphyseal capillaries were thin-

walled and dilated; the trabeculae, although present in their usual numbers, were long but thinner than ordinarily; the lines of calcium in the centers of the spicules were distinct. Large succulent osteoblasts were in mitosis; they formed several layers and large clusters in the zone underneath the cartilage and between the trabeculae. Some of the latter had broken down, and were converted into structureless eosinophilic material; particularly near the shaft, hemorrhages were noticeable. In the distal metaphysis, the bone marrow was converted into a loose fibrous tissue. The shaft contained large osteocytes with little cementing substance. The vascular canals were enlarged and filled with edematous connective tissue poor in cells, and some rudimentary osteoblasts. The periosteum was vascular and detached from the cortex of the bone, and hemorrhages were seen. The longer the vitamin C-depletion lasted, the more advanced were these lesions. After 3 weeks and later (Fig. 7), only a few small osteoblasts were present in the metaphysis and at the shaft. Few trabeculae were preserved here and they consisted of hardly more than a short calcified spur of cartilaginous matrix with a narrow rim of bony substance. They were surrounded by a few fibrocytes, elements of an edematous connective tissue which had replaced the normal constituents of the metaphysis. There were widespread hemorrhages into this fibrous tissue. Fragments of poorly ossified or noncalcified spicules and fibrinoid eosinophilic material were abundant. The severity of these lesions varied in different bones and decreased in the following order: Proximal part of the tibia, lower ribs, distal end of femur, proximal end of femur and vertebrae. The latter were involved in only one case. The shaft (Fig. 11) was further thinned out, and the enlarged haversian spaces contained edematous fibrillar tissue with engorged blood vessels. The endosteum was represented by a thin layer of fibrocytes; the periosteum was swollen in its outer layers and showed foci of hemorrhages.

Fourth Series: Vitamin C-Depleted Animals Treated with Alpha-Estradiol Benzoate

Epiphyses. Two weeks after the beginning of the experiment, the epiphyseal plates were narrow (190 μ), but regularly arranged. Hyalinization and calcification were increased over the normal, and the number of cells in a single row had dropped to 5 or 6 columnar and 3 or 4 hypertrophic cells, both cell types being greatly reduced in size. After 3 weeks, the height of the growth zones and the appearances of the cartilage were about the same as at the earlier stages (Fig. 4); the cartilaginous matrix was abundant and denser than ordinarily, but it was less calcified and looser than in the guinea-pigs receiving the

hormone and kept on the complete diet. During the fifth week, owing to a sudden pronounced swelling of the interstitial substance, the epiphyseal disks became wider. In the most distal layer of the cartilage, some eosinophilic hyaline material was present. Conditions thus were comparable to those seen in vitamin C-depleted noninjected animals after 2 or 3 weeks. Ossification of the articular cartilage was likewise diminished. There were, here and there, minute hemorrhages in the periarticular tissue. In 7 of 8 cases, the chondro-osseous junction was regular except for decreased bone formation. In the one animal showing grossly a slight rosary, conditions resembled those seen at the growth zone of the tibia, although they were less pronounced. No significant changes were noted in the vertebrae.

Diaphyses. After 2 weeks, the vascularization of the metaphysis was decreased but less so than in the estrogen-treated animal kept on the complete diet. The vessels were supported by a dense fibrillar stroma. The trabeculae were about as numerous as in normal controls. Many small osteoblasts had accumulated in close approximation to the hypertrophic cartilage cells and along the spicules, and moderate amounts of organic matrix had been deposited. Farther distally, the trabeculae were composed of solid matrix and a core of calcium salts. They were shorter, thinner, and less compact than in the animals receiving estrogen with the complete diet. On the other hand, they contained more osseous matrix than the noninjected vitamin C-depleted controls. The spicules were covered with medium-sized osteoblasts which were, in turn, surrounded by a loose connective tissue. There were no deposits of fibrinoid material nor hemorrhages as commonly found at this stage in noninjected vitamin C-deficient animals. The shaft was of the usual thickness. The endosteal osteoblasts were separated from the bone marrow by a layer of fibrocytes. The haversian canals contained engorged capillaries accompanied by dense stroma with some osteoblasts. The periosteum was dense and contained many fibrocytes, medium-sized osteoblasts, and a few osteoclasts. At the chondro-osseous junction of the ribs and in the vertebrae, the spicules were long, but thinner than in the estrogen-treated animals kept on the complete diet. After 3 weeks (Fig. 8), the metaphyseal capillaries were dilated and much loose supporting stroma was present. The trabeculae were longer than after 2 weeks, showed some interlacing, and were covered by spindle-shaped osteoblasts. They were thinner than in the estrogen-injected animals fed the complete diet, but they were more numerous and thicker than the trabeculae in the vitamin C-depleted animals receiving no hormone. The shaft was fairly thick (Fig. 12). The amount of bone present surpassed that seen in the

vitamin C-deficient, noninjected guinea-pigs. The endosteum was composed of fibrocytes and small osteoblasts. The haversian canals were wider than ordinarily but less numerous, and they were narrower than in the noninjected vitamin C-deficient animals. The periosteum was fibrillar; near the compacta, osteoblasts and osteoclasts could be identified. Again in ribs and vertebrae, the changes were less accentuated than in the tibia; there was in particular no fibrosis, hemorrhages, nor deposition of fibrinoid substance in the former. After 4 weeks, some of the thinner spicules in the metaphysis began to break down, and fragments of bone came to lie in an oblique or transverse direction. Some long spurs of calcified cartilaginous matrix with only a very thin covering of bony substance were seen. In the peripheral areas of the metaphysis, fibrous tissue had replaced the bone and some of the bone marrow. The cortex of the shaft was thinner than at earlier periods. The connective tissue in the enlarged haversian spaces and in the periosteum was edematous. Changes in ribs, femora, and vertebrae were either slight or absent. During the fifth week, the destruction of the metaphysis made rapid progress, and the findings resembled those seen in noninjected animals kept on the C-deficient diet for 3 weeks. The effects of estrogen, however, could still be recognized by the large number and the plumpness of the bony fragments present in the fibrous tissue. Frequently, the fibrinoid material showed the shape and the perpendicular arrangement of spicules and some giant cells were found in the vicinity of these structures. Also the shaft was more solid. The metaphysis of the other bones was likewise better preserved than in any of the vitamin C-depleted animals receiving no hormone.

Discussion

In Table III, the histologic findings in the various experimental and control groups are summarized. As seen from this table, alpha-estradiol benzoate decreased the growth of the epiphyseal cartilage, the vascularization of the metaphysis and shaft, and the resorption of bone, but it intensified hyalinization of cartilage and connective tissue in the long and spongy bones. As observed previously, a reversal of the estrogen effect occurred during the fifth week; it manifested itself in a resumption of growth and resorptive processes. This reversal was considered as due to an adaptation of the organism to the hormone.[1]

Vitamin C-depletion altered the proliferation of the cartilage only to a slight degree or not at all, but it caused swelling of cartilage cells and matrix, and increased the resorptive processes besides producing the lesions typical of scurvy.[6-10]

These changes appeared after 2 or 3 weeks of feeding the deficient

TABLE III

Schematic Presentation of the Changes Taking Place in the Skeletal Tissues after Four Weeks of Observation

	Cartilage			Bone (metaphysis and shaft)		Connective tissue (metaphysis and periosteum)	Vessels
	Proliferation	Hypertrophy	Regression	Amount	Resorption		
Estrogen-treated guinea-pigs	Decreased	Decreased	Increased hyalinization and calcification	Increased	Decreased	Increased, dense, hyalinized; osteoblasts small, not increased in number	Thick-walled, vascularization decreased
Vitamin C-depleted guinea-pigs	Slightly decreased	Somewhat decreased	Marked swelling	Markedly decreased	Markedly increased	Loose, edematous, acellular; osteoblasts scarce, replaced by fibrocytes	Thin-walled, congested, vascularization increased, extensive hemorrhages
Estrogen-treated and vitamin C-depleted guinea-pigs	Decreased	Decreased	Some swelling	Somewhat decreased	Somewhat decreased	Somewhat increased, decreased cellularity; osteoblasts fairly numerous, spindle-shaped	Somewhat thin-walled, vascularization somewhat decreased, some hemorrhages

diet and were most advanced in the tibia and lower ribs. The hip bone and the vertebrae, which are less subject to mechanical stress than the former, were less or not at all affected. These findings corroborated the previously established importance of mechanical factors in the production of scorbutic lesions.[8-10]

The effects of alpha-estradiol benzoate in vitamin C-depleted guinea-pigs depended upon the duration of the experiment. During the first 3 weeks, the effects of estrogen predominated, as was indicated by decreased growth processes in the cartilage and by decreased resorption of bone. At the same time, the vitamin deficiency manifested itself by a diminution in the hyalinization of the cartilage, some loosening of the connective tissue, and diminished amounts of bone, as compared with conditions in animals receiving the hormone but kept on the complete diet. After 4 weeks, and coinciding with the resumption of growth and resorptive processes seen in normally fed estrogen-treated guinea-pigs, vitamin C-depletion gained the upper hand, and during the fifth week, the scorbutic lesions progressed rapidly and with great intensity. However, the severe changes of scurvy were limited to the metaphyses of the long bones. The ribs showed only minor lesions, and the vertebrae were hardly involved at all.

The present findings may be considered from two aspects: (1) The development of scurvy as altered by the administration of alpha-estradiol benzoate, and (2) the modification of the estrogen effect by the absence of vitamin C.

Under the influence of the estrogen, growing guinea-pigs fed a vitamin C-free diet survived longer, and the skeletal lesions of scurvy appeared about 2 weeks later than in animals not receiving this hormone; but once lesions had set in, they developed in a precipitous manner. Such an effect might be obtained if the estrogenic hormone exerted a sparing effect on the vitamin C stores of the growing guinea-pig. But there is no real proof that estrogen acts in this way.

A more probable reason for the delay of the scorbutic sequence may be the inhibition of the growth of the cartilage caused by the estrogen. During the growth period, the metaphysis is constantly being remodeled by deposition of new bone and subsequent resorption of the primary spongiosa. In the absence of vitamin C, the formation of bone ceases, and the metaphysis, unable to bear the mechanical stress exerted upon it, breaks down. In the vitamin C-depleted growing guinea-pigs treated with estrogen, growth of cartilage is temporarily arrested and conditions are thus comparable to those in adult scorbutic animals. After cessation of growth, the rapid turnover of metaphyseal tissue has ceased, and the epiphysis is more securely anchored on the metaphysis

than in the growing animal. Under these circumstances, the typical sequence of scorbutic changes does not develop in the metaphysis.[11] However, after several weeks of administration of estrogenic hormone in the young guinea-pig, growth is resumed, and then the scorbutic skeletal lesions appear.

The inhibition of the resorption of bone produced by the estrogen may constitute another factor in the modified course of scurvy. In the absence of vitamin C, no new bone is formed and thus the equilibrium between deposition and resorption of osseous tissue is disturbed in favor of the latter. Obviously, the resulting atrophy of bone would be expected to be counteracted by the retardation of resorptive processes caused by the estrogen. Again, the restoration of resorption after the adaptation of the organism to the hormone will bring about a rapid appearance of the skeletal lesions of scurvy.

As to a modification of the skeletal effect of estrogen by vitamin C deficiency, inhibition of growth of cartilage—an estrogen effect—is not affected by the lack of vitamin C. On the other hand, hyalinization and calcification of the cartilage—another estrogen effect—are strikingly counteracted by the marked swelling of the cartilage caused by the vitamin C deficiency. The mode in which estrogenic hormone leads to bone formation is still under discussion. The increase of bone or bone-like tissue seen under the influence of the estrogen is not accompanied nor preceded by a proportionate increase in the number of osteoblasts. Instead of an active osteoblastic layer, a fibrous tissue is frequently found in the immediate vicinity of the excess bone. It seems difficult to understand how the spindle-shaped, apparently inert cells of this fibrous tissue could act as osteoblasts. Therefore, a decrease in the resorption of bone was thought to be at least partly responsible for the presence of increased amounts of bone seen under the influence of the estrogen; [1,4,12] furthermore, the decreased vascularization of the metaphysis and the deposition of inert hyaline material around the vessels were considered conducive to bone formation.[5] More recently, in x-ray diffraction studies in estrogen-treated mice, the material deposited adjacent to the pre-existent bone was shown not to be true bone salt—apatite, but more likely calcium carbonate.[13] This finding may be linked up with the replacement of osteoblasts by fibrous tissue as seen after administration of estrogenic hormone. Connective tissue cells may fail to differentiate into mature osteoblasts and may thus be unable to form true bone, but they may yet to some degree be capable of promoting the precipitation of less complex calcium salts from the serum. This phenomenon might be interpreted as bone formation of a more primitive type.[14] The results of the present investigation sug-

gest that besides connective tissue cells and calcium salts, a third element, namely, an intact interstitial matrix, is a prerequisite for the production of this "primitive bone." After administration of alpha-estradiol benzoate, less bone-like tissue was found in vitamin C-deficient animals than in those fed the complete diet. In view of the established effects of vitamin C on the intercellular matrix, it may be assumed that the inadequacy of the latter accounts for the failure of the estrogen to exert its usual effects on bone.

The effects of the estrogen on the vessel walls are likewise counteracted by ascorbic acid deficiency. Actual hyalinization of the capillary walls or deposition of hyaline material in the perivascular connective tissue as seen in mice and birds is difficult to demonstrate in the guinea-pig. However, in the latter species, the vessel walls are thickened after treatment with alpha-estradiol benzoate. Both the metaphyseal and periosteal blood channels are affected in this manner. The increased thickness of the vessel walls and the associated loss of elasticity are at least partly responsible for a decrease in the resorptive processes. In vitamin C-depleted guinea-pigs injected with estrogen, these vascular changes were not observed, and for this reason resorptive processes could take place in a more normal fashion. The inability of the organism to form cementing substances in the vessel wall in avitaminosis C may account for the absence of this estradiol effect. Again, as in the case of the cartilaginous ground substance, this interference is probably a true antagonistic effect of two factors acting on the same constituent of the intercellular matrix.

Summary

In growing guinea-pigs, the absence of vitamin C modifies the skeletal effects of alpha-estradiol benzoate as follows: The calcification and hyalinization of the ground substance caused by the hormone are counteracted, while the suppression of growth of cartilage produced by estrogenic hormone is not affected. Excessive amounts of bone in the metaphysis found in normally fed animals under the influence of estrogen are absent in avitaminosis C. This is probably due to the inability of the intercellular matrix to be converted into bone, if vitamin C is lacking. Likewise, thickening of the vessel walls seen after treatment with estrogen does not take place in hormone-treated vitamin C-deficient animals. The onset of the scorbutic sequence in the metaphysis of the long bones is retarded and its appearance in ribs and vertebrae is counteracted by the administration of estrogenic hormone to vitamin C-depleted animals.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

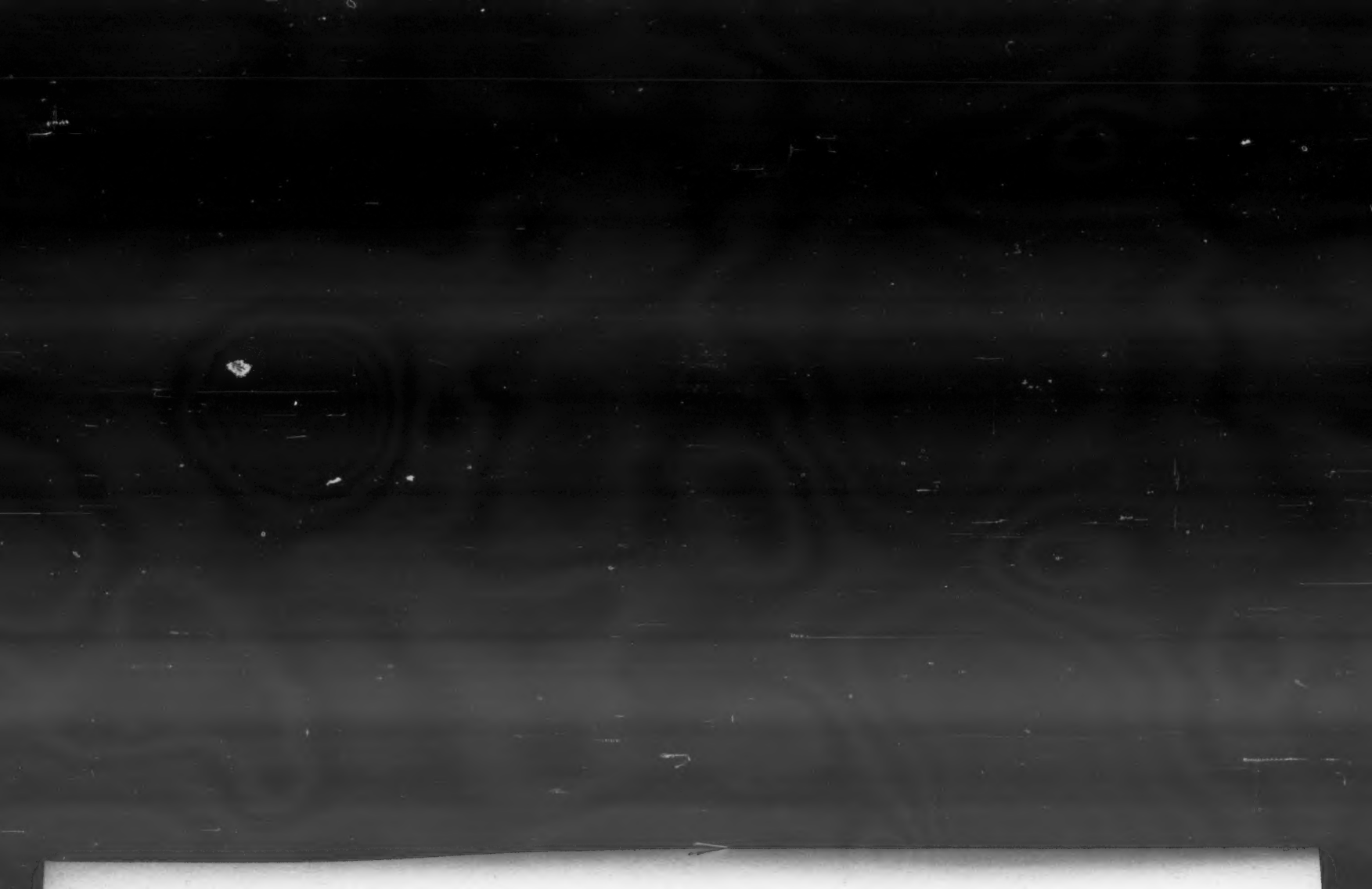
PLATE 167

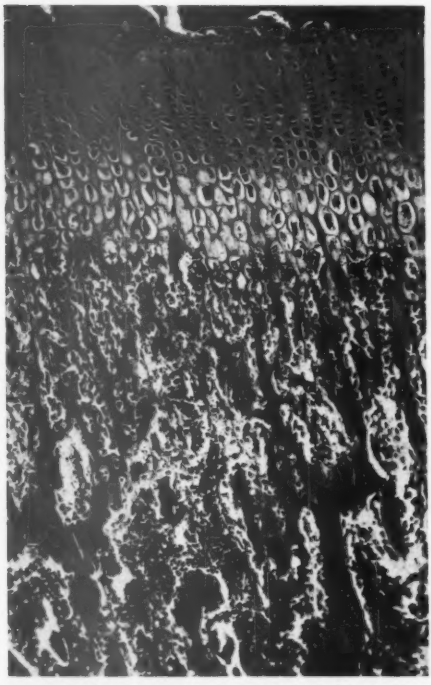
FIG. 1. Section through the growth zone at the upper end of the tibia of a male guinea-pig, whose weight had increased from 185 to 305 gm. during 3 weeks of observation. The epiphyseal plate and the metaphysis show a regular configuration. × 95.

FIG. 2. Section through the growth zone at the upper end of the tibia of a male guinea-pig which received a total of 150 mg. of alpha-estradiol benzoate over a period of 3 weeks. The weight of the animal had increased from 182 to 351 gm. during the time of observation. The epiphyseal plate is narrowed and heavily calcified, the cartilage cells are smaller, and the trabeculae, particularly in their distal parts, are denser and thicker than in Figure 1. × 95.

FIG. 3. Section through the growth zone at the upper end of the tibia of a male guinea-pig kept on a vitamin C-free diet for 3 weeks. The initial weight of the animal was 190 gm., the final weight 188 gm. The pattern of the epiphyseal plate is irregular and the cartilaginous matrix is loose. "Fibrinoid" material is seen in the distal layers of the cartilage and in the abundant loose fibrous tissue of the metaphysis; the trabeculae are destroyed. × 95.

FIG. 4. Section through the growth zone at the upper end of the tibia of a male guinea-pig kept on a vitamin C-free diet, which received a total of 150 mg. of alpha-estradiol benzoate during this period. Its initial weight of 184 gm. had increased to 286 gm. during the period of observation. The epiphyseal plate is narrowed and intact, the cartilage cells are smaller than in Figure 1, and there is increased hyalinization. The metaphysis contains well formed but slender trabeculae. No "fibrinoid" material and no fibrous tissue are found in the metaphysis. × 95.





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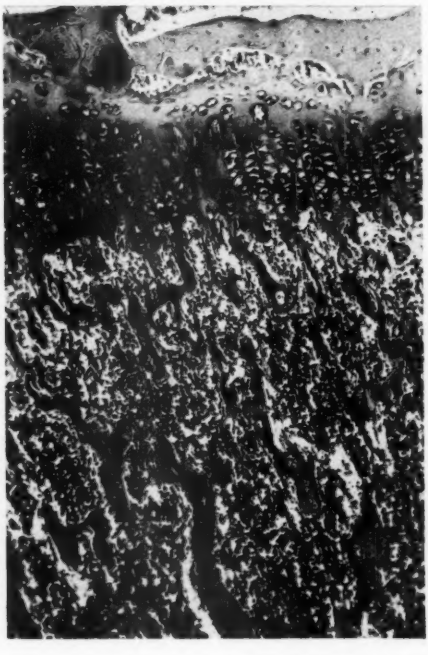

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PLATE 168

FIG. 5. An area of the metaphysis of the tibia in Figure 1. The hypertrophic cartilage cells are being eroded by engorged capillaries, and many large polyhedral osteoblasts have been laid down along the trabeculae. × 285.

FIG. 6. An area of the metaphysis of the tibia shown in Figure 2. Two hyalinized plugs of degenerated cartilage are seen between the hypertrophic cartilage cells. The latter are smaller and the vascularization is decreased as compared with Figure 5. The trabeculae are long and contain unopened cartilage cells. They are covered by osteoblasts which are smaller and more spindle-shaped than in Figure 5. × 285.

FIG. 7. An area of the metaphysis of the tibia shown in Figure 3. The cartilage cells are distorted and cells of hypertrophic type are absent. The distal part of the cartilage and the metaphysis contain fibrinoid material. There is increased vascularization and enlargement of the capillaries. The primary trabeculae are destroyed. × 285.

FIG. 8. An area of the metaphysis of the tibia shown in Figure 4. The epiphyseal cartilage cells are preserved and better developed than in Figure 7. There is less hyalinization than in Figure 6. Bony spicules are present, but they are shorter and thinner than in Figures 5 or 6. Vascularization is decreased as compared with Figure 7, but increased as compared with Figure 6. Numerous, but small, osteoblasts cover the trabeculae. × 285.

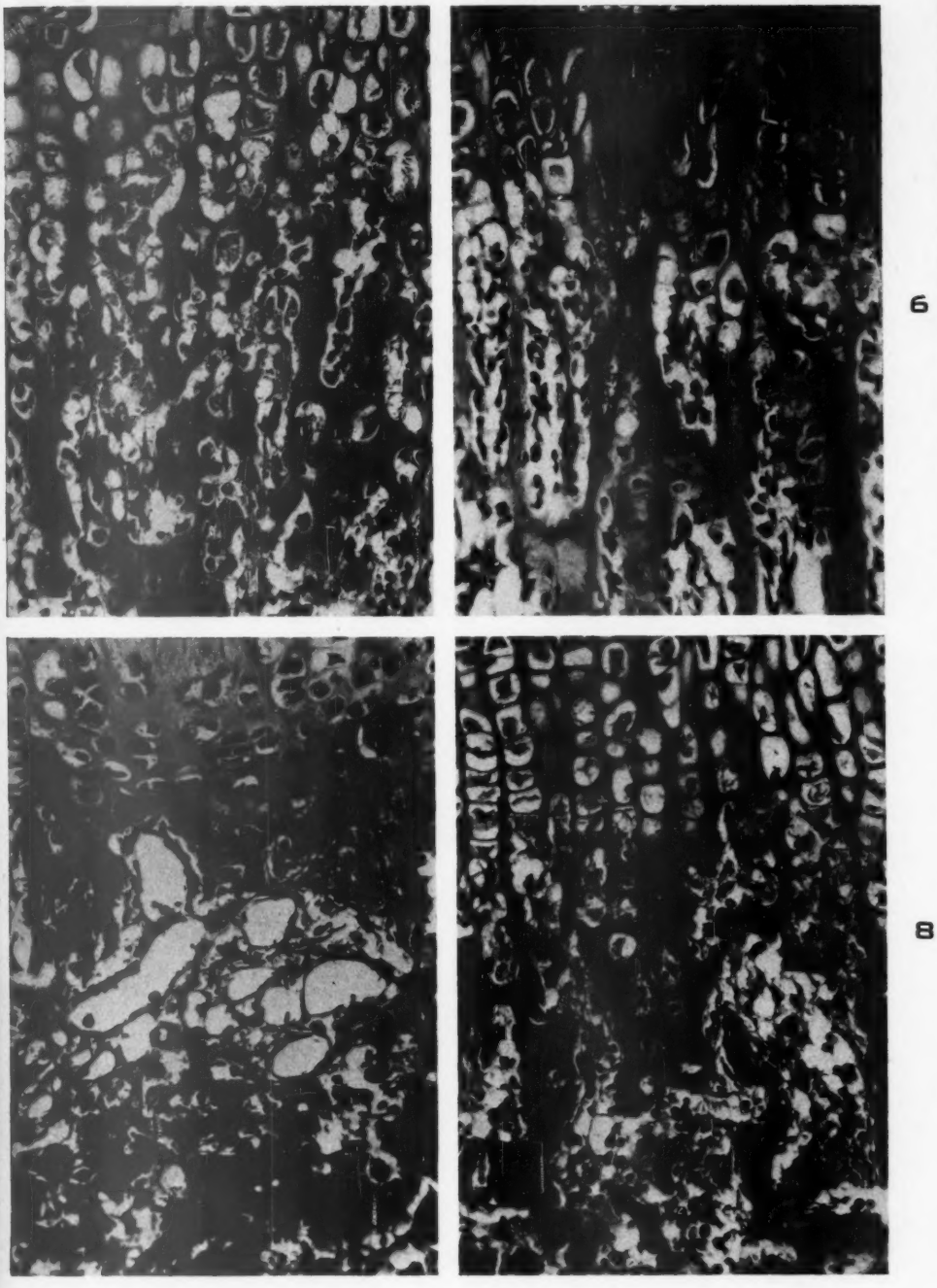

PLATE 169

FIG. 9. Section through the cortex of the tibia shown in Figure 1. The endosteum consists of many large osteoblasts; the periosteum is loose and contains osteoblasts and osteoclasts. × 95.

FIG. 10. Section through the cortex of the tibia shown in Figure 2. Osteoblasts at the endosteum are spindle-shaped and scantier than in Figure 9. The vascularization is decreased, and the osteocytes are small. The outer layer of the shaft is smoother than in Figure 9 and does not contain lacunar grooves with osteoclasts. The periosteum is dense and somewhat less vascular. × 95.

FIG. 11. Section through the cortex of the tibia shown in Figure 3. The cortex is markedly thinned out, the endosteum consists of spindle cells, the vascular canals are greatly enlarged and contain loose connective tissue poor in cells, and the periosteum is edematous and detached from the cortex. × 95.

FIG. 12. Section through the cortex of the tibia shown in Figure 4. The cortex is thicker, the vascular canals are less engorged, the connective tissue is more cellular and denser, and the periosteum is less edematous than in Figure 11. × 95.

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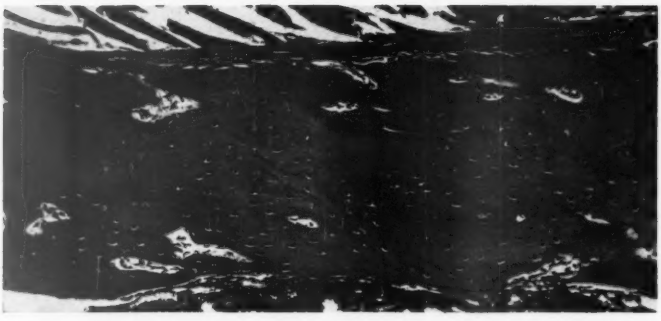

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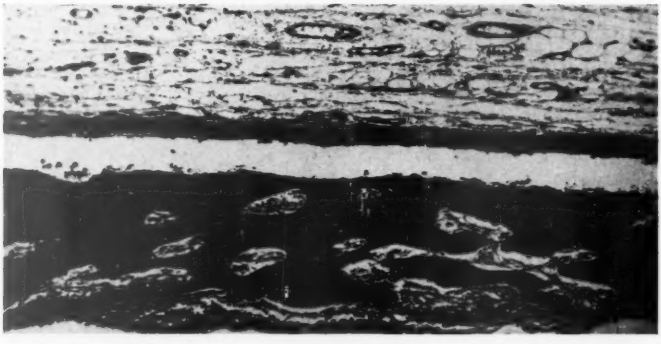

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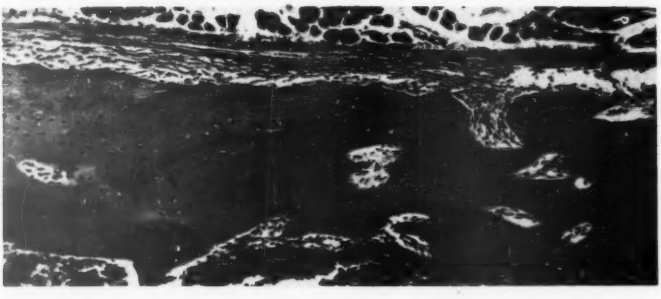

CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE
IV. Morphologic Changes Associated with the Death of Cells in Vitro and in Vivo *

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In this paper changes in dying and dead cells as viewed with the phase microscope (PM) are described. The technic and the material used in these experiments have been described in a preceding paper.[1]

Death of an individual means generally "cessation or extinction of life," whereas cell death is interpreted as "complete degeneration or necrosis of cells" (Dorland[2]). This purely morphologic description of cell death is not satisfactory. Therefore, a method has to be found by which it is possible to test the viability of a cell, thus giving the morphologic statement the necessary biologic background.

Observations

When living cells are suspended in a physiologic medium (saline, buffered glucose Ringer solution (BGR), homologous serum) at 37°C. for from 1½ to 3 hours, the nuclei exhibit a change described as the intermediate stage[3]: the nuclear membrane becomes thicker and more irregular, the chromatin network is replaced by numerous black, irregular dots of variable size, and the whole nucleoplasm appears somewhat darker (Figs. 1 and 2). In this stage, the cilia of ciliated epithelium gradually move more slowly, and finally stop; but they vibrate again when fresh physiologic saline solution is added to the suspension. Later, more and more nuclei show brilliant nucleoli (Fig. 3), and then the nuclear membrane also becomes brilliant and double-contoured (Fig. 4). The chromatin network of such cells is very plump, some of its condensations (karyosomes) are brilliant, and the nucleoplasm again appears lighter. The protoplasm has shrunk slightly, the mitochondria are medium-sized, and the storage granules[4] are enlarged (Fig. 5). This can be called the brilliant type of nuclear change.[3] These cells, particularly the nucleus and the storage granules, are stained deep red by neutral red in a strength of 1:10,000. Suspensions which have been at 37°C. for 6 hours contain, exclusively, cells with brilliant nuclei.

At room temperature, the cilia of large pieces of tracheal epithelium of frogs and mice still move very fast after 6 hours. Later, they stop

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moving in one cell after the other. At the same time, the first signs of the brilliant type of nucleus appear. After 24 hours, this suspension contains about 70 per cent of cells with brilliant nuclei, and no ciliary movement can be seen after the addition of fresh saline solution.

A cell suspension, after having been in the icebox at 6°C. for 24 hours, shows about 5 per cent of cells with brilliant nuclei, and the storage granules are strikingly small, when compared with those of suspensions stored at higher temperatures. When this suspension is transferred to a water bath at 37°C., the cells show the different stages mentioned above within a much shorter time.

Cells in the mitotic phase contain brilliant, irregular chromosomes after being in the suspension at 37°C. for 30 minutes only. After 1 hour, the chromosomes of the cells in mitosis are no longer visible (see study III [3]). The mitotic cells with brilliant chromosomes contain relatively large, black protoplasmic granules, and the perichromosomal halo is less distinct than in fresh mitotic cells.

The nuclei of cells situated in the centers of large cell clumps and of tissue fragments turn brilliant long before the single cells and the cells on the edges of clumps.

The cilia of cells with brilliant nuclei never move, and the movement cannot be revived by any means. This was interpreted as indicating that cell death had occurred. In order to obtain a second biologic proof of the death of cells with brilliant nuclei, the following experiment was carried out. Three sterile cell suspensions (C_3H sarcoma, Gardner's lymphosarcoma, and a spontaneous breast tumor of the mouse) were kept in the icebox for 24 hours. Then, after observation with the PM had shown that about 6 per cent of the cells had brilliant nuclei, 0.5 cc. portions of each suspension were injected subcutaneously at different sites into 4 C_3H mice. Another series of 4 C_3H mice were injected with the same amount of similar suspensions, which had been kept at 37°C. for 24 hours; a PM examination before the injection showed that all of the cells of these latter suspensions contained brilliant nuclei.

Examination of the tumor growth by palpation on the seventh day and by autopsy on the tenth day revealed good tumor growth in every site and in every animal of the first series, whereas the tumor cells of the second series, exhibiting brilliant nuclei, did not grow in any animal.

In order to investigate the influence of high temperature on cells, suspensions of Brown-Pearce carcinoma cells in thin-walled test tubes were placed in water baths of different temperatures. Samples of these heated suspensions, the bacteriologic sterility of which was maintained

carefully, were investigated after various intervals of time, and the approximate percentage of cells containing brilliant nuclei was determined. At 37°C. the majority of the cells showed nuclei in the intermediate stage after 2 hours. The mitochondria, and particularly the storage granules, were enlarged. About 15 per cent of the cells contained brilliant nuclei. After 4 hours at 37°C., 54 per cent, and after 5 hours 82 per cent of the cells in the suspension exhibited the brilliant type of nuclear change. One hour later, the suspension contained only a very few cells without the brilliant change of the nucleus. About the same percentage was found when normal rabbit serum was used instead of physiologic saline solution.

A temperature of 50°C. brings about the typical brilliant change of the nuclei in about 32 per cent of the cells after 2 minutes. At this temperature, the mitochondria first enlarge, and later decrease in size. The intermediate stage of nuclear change appears in numerous cells of this suspension. Figure 6 demonstrates such a cell, the nucleus of which turned brilliant 6 minutes after this picture was taken (Fig. 7).

Boiling water immediately produces the brilliant type of nucleus in every cell. The mitochondria and the cellular membrane disappear, and the protoplasm becomes net-like. The cellular membrane also disappears after 10 minutes at 65°C., and after 5 minutes at 75°C. About 10 per cent of the cells exhibit this alteration even after 2 minutes at 75°C., after 5 minutes at 65°C., and after 10 minutes at 56°C.

After 20 minutes the storage granules are slightly enlarged at 37°C. whereas they are small at 45°C. and at 56°C. They disappear after 10 minutes at 65°C., after 5 minutes at 75°C., and immediately in boiling water.

That alcohol, acetone, formalin, and acids also bring about the brilliant type of nucleus has already been described.[3] It may be added that the ciliary movement stops in every case as soon as the chemical reaches the cells, and that it cannot be revived by replacement of the chemical by physiologic saline solution. This is true even for the temporary intermediate stage of nuclei in potassium bichromate. The only differences between cells that died spontaneously in suspensions and those killed by formalin, etc., are the thickness of the brilliant nuclear membrane, which is slightly less in the former than in the latter, and the fact that cells that died spontaneously are stained brighter red by neutral red in a strength of 1:10,000 than are the others.

Distilled water produces another type of nuclear change. The nuclei become slightly swollen and the nucleoplasm appears hazy, homogenous, and gray, whereas the nuclear membranes and the nucleoli remain

distinctly visible. Many hours later, the nuclei may fade, or even disappear (Fig. 8). The cellular membranes and protoplasmic granules of both types disappear much later. In distinction from the brilliant type this is called the hazy type.[3] Distilled water does not kill the cells within a short time. The cilia still move very fast, and thus turn the cells over and over, even when the nuclei have been hazy for a few minutes (Fig. 9). Then, the cilia stop vibrating in one cell after another, but not earlier than 12 minutes after the addition of the distilled water (Fig. 10). When distilled water is replaced by physiologic saline solution at this moment, some of the cells renew their ciliary movement, but in many of the cells nothing happens. In distilled water blister formation [1] continues and even starts in cells with hazy nuclei, whereas this phenomenon never occurs in cells with brilliant nuclei. The same is true for the enlargement of the storage granules.[4]

Cilia stop moving when molar NaCl replaces the physiologic medium and the nuclei turn hazy.[3] Vibration cannot be revived even if the molar NaCl is replaced by physiologic saline solution just after the nuclei have turned hazy. Cells, the nuclei of which have been brilliant before the addition of molar NaCl, shrink and turn yellow when reached under the coverslip by molar NaCl; the nucleus is no longer visible in such mummy-like cells. There is no similarity between this change and the hazy type, which is produced by the action of molar NaCl on living cells. Formalin (Fig. 11), alcohol, acetone, and acids have the same effect on dead cells.

In order to make absolutely sure about the viability of cells in molar NaCl and in distilled water, a biologic test was used. A bacteriologically sterile suspension of Brown-Pearce carcinoma cells in BGR was prepared and kept at room temperature for 15 minutes. Then, part of it was injected into rabbits (suspension A, control), after it had been noticed with the PM that practically all of the cells were unchanged. From the rest, suspensions B, C, and E were prepared. One and one-half cc. of the original suspension was mixed with 4.4 cc. of distilled water (suspension B); under the PM all of the cells were enlarged, the mitochondria were vesicular, and the nuclei were hazy. Ten minutes later, this suspension was restored to isotonicity by adding an adequate amount of molar NaCl. Under the PM these cells looked absolutely normal. Suspension C (4.5 cc. of BGR mixed with 1.5 cc. of the original suspension) served as a second diluted control. Under the PM all of the cells were found to be as well preserved as in the original suspension A. Suspension D was obtained by pressing the minced tumor pieces through a metal sieve into molar NaCl. All of the nuclei of this suspension were hazy under the PM. An attempt

was made to restore this suspension to isotonicity by means of distilled water (suspension E) but on account of the considerable dilution the suspension contained only a few intact cells, all showing nuclei of the hazy type.

The different suspensions were implanted into 3 adult agouti rabbits. Each animal received intramuscular injections of 1 cc. at six different sites (upper extensors of all four legs, flexors of the thighs): suspension A in three sites; B, C, and D, each in one site. One animal received an injection of suspension E instead of D.

After 9 days, the growth of the tumors was determined by palpation. A tumor of about 3.5 cm. in length was found at each site where suspensions A, B, and C had been injected. At the site of the other injections, no tumor could be palpated. Autopsies confirmed these findings in all animals.

From this experiment it became evident that the Brown-Pearce carcinoma cells were killed by molar NaCl, but that they survived the action of distilled water, although their nuclei had been hazy for at least 15 minutes.

The hazy type of nuclear change also appears in cells of a suspension which remains under the coverslip for several hours without being protected from evaporation (Fig. 12). When fresh physiologic saline solution or BGR is added on the edge of the coverslip, the nucleus turns brighter, the chromatin network as well as the nucleoli reappear, and the cilia start moving again. A somewhat similar alteration is produced by pressure on the coverslip, but in that case the nuclear and cellular membranes are destroyed and the mitochondria disappear, whereas the storage granules remain unchanged (Fig. 13). No signs of life can be seen in such cells, and cilia never move again after the nuclei exhibit this change.

In fresh suspensions of a partly necrotic tumor cellular changes of two further types appear: pyknosis and necrosis. The nuclei of pyknotic cells are very compact, dark, and somewhat granular under the PM. The protoplasmic granules are no longer visible, and the whole cell is more refractive, shiny yellow, and slightly shrunken. Necrotic cells (see Fig. 22 of study II [4]) are very small, irregularly shaped, and shiny yellow, with the exception of rare nuclear fragments which are dark, round particles, 2 to 6 μ in diameter. There is no nucleus to be seen. Every necrotic cell is stained deeply and entirely when neutral red (1:10,000 in physiologic saline solution) is added to the suspension. Such cells do not show any reaction to ammonia and to the other chemicals mentioned above. The transformation of cells containing brilliant nuclei into necrobiotic or necrotic cells does not take place

before the cells have been in the suspension for from 2 to 4 days. The nuclei disappear by karyorrhexis or karyolysis. In very fresh suspensions of partly necrotic tumors, intermediate stages between brilliant nuclei and pyknotic or necrotic cells occasionally are seen.

Discussion

Cowdry [5] defined death as "the disorganization of living matter, which makes permanently impossible all vital phenomena." One of these vital phenomena, which can be observed under the PM, is the movement of the cilia of the ciliated epithelium of the frog's pharynx. This motion is an indication of the viability of a cell, whereas the lack of ciliary movement suggests the death of a cell, provided that the restoration of the suspension medium to normal conditions does not engender further movement of the cilia. A second and more trustworthy indication of viability is the multiplication of malignant tumor cells after their injection into susceptible hosts. As a matter of fact, the experiments mentioned above show that the same adverse surroundings under which cells suffer loss of the vegetative function of ciliary movement also cause loss of the ability of malignant tumor cells to grow in susceptible hosts.

Furthermore, these studies with the PM demonstrate that various morphologic alterations of the nuclei develop simultaneously with the loss of the viability of cells. Cells which have been in a physiologic medium for several hours first show the intermediate stage of nuclear change,[3] and then turn brilliant.[3] This increase of the nuclear refractivity and the appearance of a marked nuclear membrane are considered by Lewis and McCoy [6] as typical signs of cell death. The biologic test mentioned above confirms this assumption. The viability of cells showing nuclei in the intermediate stage could not be tested, since suspensions never contain this cell type exclusively.

It was shown [4] that the storage granules in dying cells probably carry waste products of the cells. The enlargement of the storage granules is distinctly accelerated at relatively high temperatures, considerably delayed at low temperatures, and even totally suppressed in the icebox. This suspended or delayed accumulation of waste products at low temperature (vitrification) may be one explanation of the prolonged survival time of the cells, and it is known that cells can be revived after having been in a state of total vitrification for weeks and even months.

The beginning of the brilliant type of nuclear change indicates that cell death has already occurred. Baker [7] and Bradley [8] showed that the intracellular pH becomes acidic immediately after death, and the

experiments with acids [3] demonstrated the same qualitative effect of acids on the nuclear elements as observed in spontaneous death. The slight qualitative difference between cells which died spontaneously and those which were artificially killed, which consists in a smaller width of the nuclear membrane in the former, is presumably a consequence of the higher precipitative potency of these chemicals.

It has been known for a long time that the nuclei of dead cells in suspensions are stained diffusely by neutral red, etc., whereas they are not stained during life. Schrek [9] stated that formalin-killed cells do not take eosin (1:2,000), whereas cells which die spontaneously are stained red. In my experiments when eosin was used one could observe a slight delay in the staining of formalin-killed cells and a paler color. Dead cells of both types were well stained by neutral red in a 1:10,000 solution.

From the observations on the death of cells by heat it is clear that the percentage of dead cells depends not only on the temperature, but also on the time during which the hot solution acts upon the cells. Strong heat, even 75°C., apparently does not kill all of the cells of one particular type in a suspension at the same moment. It is conceivable that this is due to differences in the sensitivity of the single cells, depending on their age and their stage of degeneration previous to the heat experiment.

The hazy type of nuclear change, which is produced by distilled water, molar NaCl, ammonia, alkali, and mechanical destruction of the cells, is not a reliable sign of death since cells which show the hazy type of nuclei caused by distilled water can be revived. On the other hand, when the hazy change of nuclei is caused by any other of the above-mentioned agents, these cells cannot be revived, and should be considered dead. It is also of interest that the appearance of the hazy type of nucleus proves that the cells have been living before the agent acted upon the cells, because previously dead cells are converted into yellow mummies when exposed to these agents, and do not show hazy nuclei (Fig. 11).

Contrary to the opinion of Lewis,[10] blisters are not a sign of death. In fact, the initial formation, or a further growth of blisters in dead cells, has not been seen, and the majority of dead cells do not contain blisters. Cells which have already formed blisters may retain them after death, but the formation or the further enlargement of already existing blisters is a reliable sign of life.[1]

The movement of the cilia is another very reliable indication of the viability of cells. When the cells die, it stops and the nuclei turn brilliant simultaneously; the movement can never be revived in such cells.

In cells containing hazy nuclei produced by the action of distilled water or molar saline solution, the ciliary movement corresponds with the outcome of the biologic test. In distilled water, the cilia move for a time, or if this movement has stopped it usually can be revived by the action of normal saline solution. Accordingly, malignant tumor cells treated with distilled water for a limited time do not lose their ability to grow in susceptible hosts. In molar saline solution, on the other hand, the ciliary movement stops irreversibly, and the cells do not grow in hosts.

With respect to the protoplasm of dying cells, the descriptions offered by Lewis [10] and Lewis and Lewis [11] are somewhat different from the observations reported above. These authors mentioned that the mitochondria, as well as the "degeneration granules," and the vacuoles disappear, and that their disintegration is followed by granules of a new type, called "death" or "d-granules" by Lewis.[10] However, the appearance of granules of a new type was never observed in the experiments mentioned above, and the mitochondria did not disappear until the nucleus broke down. The storage granules were still well preserved, but very much enlarged, even when the cells disintegrated in physiologic saline solution at 37°C. after 1 day. The "death granules," therefore, are likely to be identical to the enlarged storage granules. On the other hand, one has to take into consideration the fact that the observations of these authors are based on a material different from that used in my experiments.

The degenerative protoplasmic alterations, as seen in cells in old suspension, and the intermediate stage of nuclear change in such cells, remind one very much of cloudy swelling (see, for instance, the description given by Karsner [12]). Water-intake is considered to be the first step leading to cloudy swelling. In cell suspensions it manifests itself by blister formation,[1] but in tissues the entire cell becomes swollen. Karsner suggested that acidification of the protoplasm could be the reason for these changes. This opinion corresponds to the explanation of the final stages of cell death given above. The fact that cells in the mitotic phase die much sooner in suspensions than do resting cells is a further proof of the increased sensitivity of cells during mitosis.

The mummy-like cells, produced by the action of various chemicals upon already dead cells, are generally called necrotic if viewed in microscopic sections, and, as a matter of fact, cells of necrotic tumor areas show a similar structure to these mummies when viewed with the PM. The small structural differences between genuine necrotic cells in tissues (*in vivo*) and mummy-like cells which develop under the influ-

ence of fixatives from fresh dead cells (*in vitro*) are likely to be based upon differences in the manner of action of the protoplasmic autolytic enzymes and of the chemicals added to the suspension, both of them altering the already dead cells. According to Baker,[7] only the cleavage of the nucleoproteins into amino acids by enzymes brings about the typical structure of necrotic cells. Therefore, the disappearance of the nuclei in stained microscopic sections of necrotic areas does not signify more than the fact that the resorption of dead cells has already started. Recent cell death as such cannot be recognized in microscopic sections, since all of the cells are killed anyway during the process of fixation. Small wonder that by means of the ordinary microscope one cannot recognize any alterations in sections of very fresh infarcts of the myocardium, etc. It can be expected that the PM will be of valuable practical help for future studies in this field, because it discloses even the earliest changes indicating death.

What is the significance of pyknosis and necrobiosis in general? It already has been stated that the development of neither pyknosis nor necrosis can be observed in short-term experiments *in vitro,* provided that no chemicals are added; the further changes of dead cells in suspensions consist of a decrease in nuclear size, and later of karyorrhexis, a very slow disintegration of the storage granules (Fig. 14), and disappearance of the mitochondria. In fresh cell suspensions, it is possible to find the various stages of nuclear disintegration, which has taken place *in vivo*. The earliest stage is the brilliant type; then follow pyknosis, karyorrhexis, and karyolysis (necrosis). Accordingly, pyknosis—one of the various signs of necrobiosis—as well as necrosis itself, are stages in a consecutive series of secondary changes in dead cells.

Summary

The phase microscope makes possible a method by which the cytology of dying and dead cells can be studied without interference by the effects of fixation and staining.

The most conspicuous alterations indicating cell death occur in the nucleus, as described in a preceding paper.[3] One can distinguish between a *brilliant* and a *hazy* type. A third alteration, the temporary *intermediate stage,* is produced by 3 per cent potassium bichromate; it can be seen also in old cell suspensions. The brilliant type, caused by over-age of the suspension, heat, fixatives, or in other ways, is a reliable sign of death. The viability of cells exhibiting the intermediate stage of nuclear change could not be tested. The hazy type following the action of distilled water does not, as such, prove cell death, because such cells in distilled water can be revived. With molar NaCl,

ammonia, alkali, and mechanical crushing of cells, hazy nuclei indicate cell death.

In addition to the nuclear structure, the ciliary movement is an excellent morphologic indicator for the viability of cells; the movement stops irreversibly and simultaneously with the appearance of nuclear signs of death. The same is true for the new formation and the further growth of blisters. Cells of transplantable tumors do not grow in susceptible hosts if they show the above-mentioned morphologic signs of death.

Mitotic cells die much sooner in suspensions, and they are generally more sensitive to artificial changes of the surrounding milieu than are cells in the resting phase.

Necrotic and necrobiotic cells are dead cells that show alterations caused by the action of autolytic intracellular enzymes in addition to the nuclear and protoplasmic changes mentioned above. Necrosis and necrobiosis occur only after 2 to 4 days *in vitro* under the experimental conditions herein described.

It is conceivable that cloudy swelling of cells *in vivo* is identical to the first stage of degeneration observed in cells in suspensions.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 170

FIG. 1. Brown-Pearce carcinoma cells which have been at 37°C. for 90 minutes. There are large blisters in both cells on the left, and some vacuoles in the right upper cell. The chromatin network begins to appear more granular in the two cells on the left, when compared with the upper cell. PM. × 1400.

FIG. 2. Brown-Pearce carcinoma cells, which have been in a suspension at 37°C. for 2½ hours. The two cells in the left upper quadrant show the intermediate stage of nucleus; in the nucleus below, this change is just beginning. PM. × 1400.

FIG. 3. Dying Brown-Pearce carcinoma cells, which have been at 37°C. for 4 hours. The chromatin appears more like spots than in the form of a network, the nuclear membranes are more irregular and thicker than in normal cells, and the nucleolus of the lower left cell is already brilliant. PM. × 1400.

FIG. 4. C3H sarcoma cells, later stage of death (4½ hours). The two cells on the right are dead, showing an irregular, double-contoured, brilliant membrane and brilliant nucleoli. The cell on the left shows a slight wrinkling of the nuclear membrane, and enlarged chromatin condensations. Of note are a large blister and greatly enlarged storage granules of the upper of the two dead cells. PM. × 1400.

FIG. 5. Brown-Pearce carcinoma cell, last phase of death (6 hours), showing shrinkage of the nucleus. A previously existing blister has not disappeared. PM. × 1400.

FIG. 6. Brown-Pearce carcinoma cell, which has been at 50°C. for 2 minutes. The nucleus is slightly shrunken, the nuclear membrane is irregular, the chromatin network is dense, and the nucleoplasm has become cloudy. PM. × 1400.

FIG. 7. The same cell as shown in Figure 6, 6 minutes later. The nuclear changes are still more distinct, the nuclear membrane and the nucleolus are brilliant, and the storage granules are enlarged. PM. × 1400.

FIG. 8. Late phase of the hazy changes. Ghost cells, with but little recognizable cellular structure, are seen. PM. × 1400.

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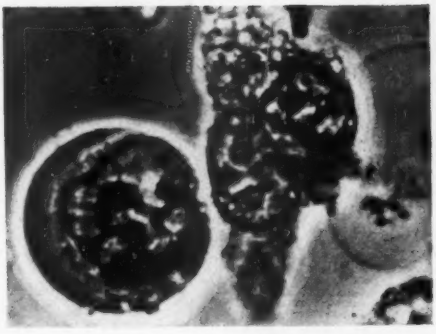
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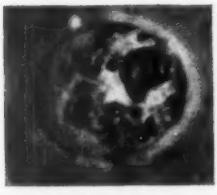
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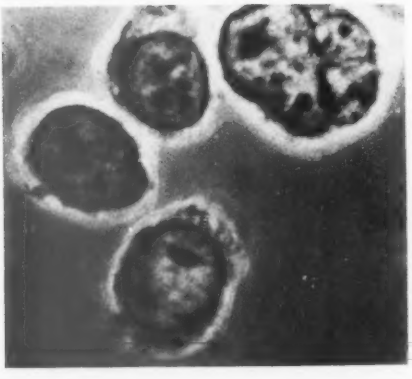
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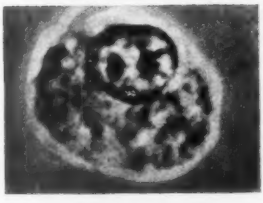
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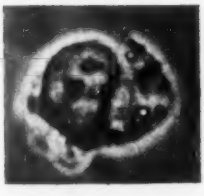
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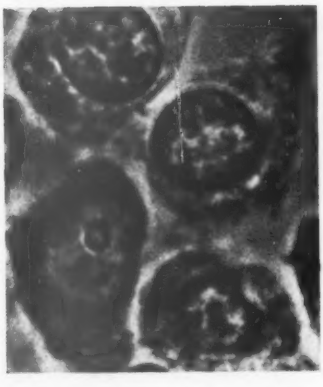
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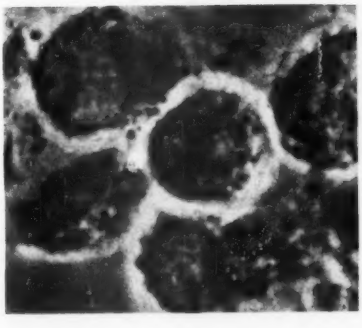
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Zollinger Phase Microscopy, Cellular Death

PLATE 171

FIG. 9. Ciliated epithelial cell of the frog pharynx after having been in distilled water for 3 minutes. The cilia move so fast that the cell constantly turns around and, therefore, appears indistinct. The nucleus is already hazy and enlarged; the nucleolus of the cell on the right is visible. PM. × 1400.

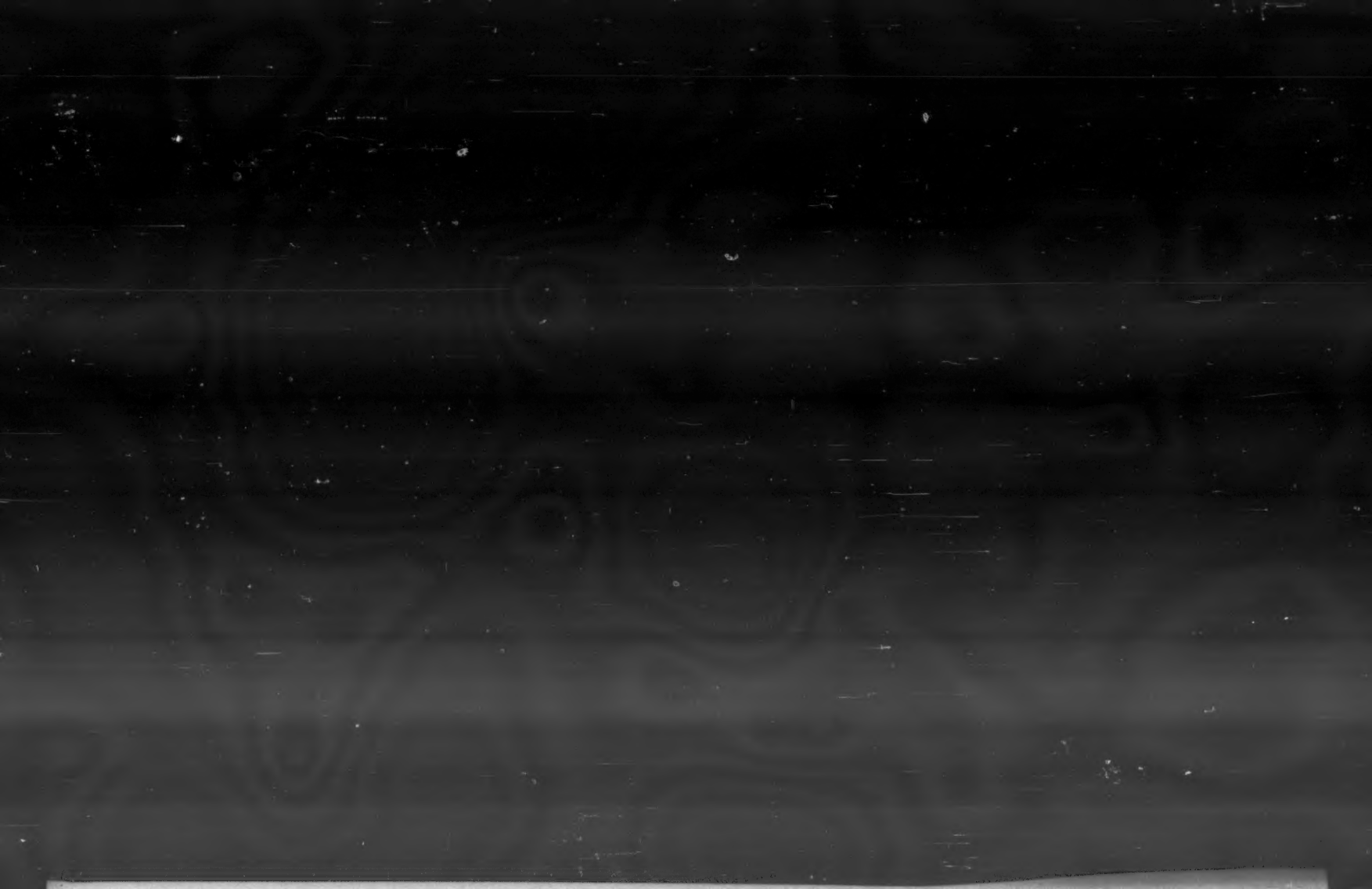
FIG. 10. The same cell as shown in Figure 9, 8 minutes later. The cilia no longer move; a large blister has appeared; vacuoles have formed; and the nuclei are hazy. PM. × 1400.

FIG. 11. The effect of 10 per cent formalin upon living and dead cells. The nucleus of the uppermost cell was already brilliant before formalin was substituted for the physiologic saline solution; the nuclei of the other cells are unchanged. The formalin has just reached the cells: the nuclei of cells previously living show the intermediate stage, whereas the dead cell is shrunken, and has become more refractile. PM. × 1400.

FIG. 12. Frog kidney cells after the same sample of the suspension was observed under the coverslip for 45 minutes. The cells are beginning to change their appearance because of evaporation of the suspension fluid. The nucleoplasm of the two cells on the left has become hazy and almost homogenous, whereas the nucleoli are unchanged. The storage granules and the mitochondria are enlarged. PM. × 1400.

FIG. 13. Brown-Pearce carcinoma cells adjacent to a crushed area caused by local pressure on the coverslip. The nuclei are hazy, but, with the exception of the cell on the left, they are distinctly outlined; the nucleoli have disappeared. The granules are decreased in number, and the protoplasm contains numerous small vacuoles. PM. × 1400.

FIG. 14. Brown-Pearce carcinoma cell, which was observed for 36 hours after spontaneous death. The nucleus is darker and smaller than in recently dead cells. The majority of the storage granules have disappeared and the mitochondria are no longer recognizable. PM. × 1400.



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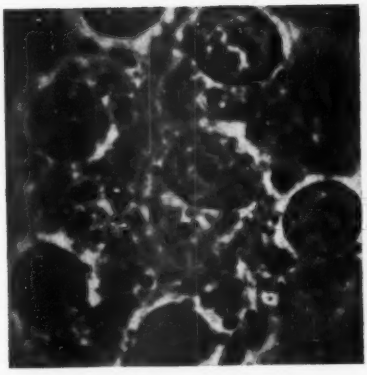

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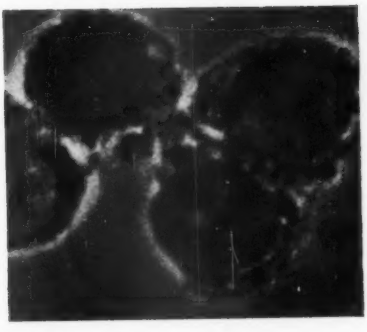

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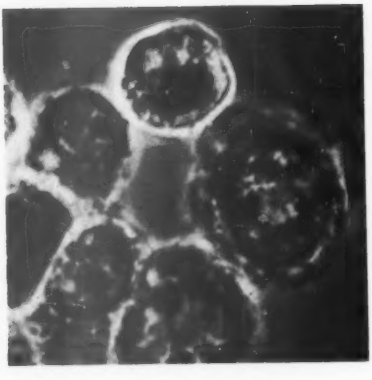

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Zollinger

Phase Microscopy, Cellular Death

CYTOLOGIC CHANGES IN THYMIC GLANDS EXPOSED IN VIVO TO X-RAYS *

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In previous work,[1-4] cellular suspensions derived from the thymic glands of rabbits were treated with x-rays and were studied by the method of unstained cell counts, by dark-field microscopy, and by histologic sections. It was found that x-rays killed the thymic cells after a latent period of 3 hours or more. During the latent period, many of the viable, irradiated cells developed single or multiple structures which were visible on dark-field illumination and which were termed primary vacuoles. In histologic sections of the suspension, the primary vacuoles were seen to be intranuclear. Nearly all of the irradiated cells were dead after 24 hours of incubation at 37°C. Dark-field examination showed that the dead cells were small and round and had single, fairly large, dark, partly encapsulated structures which were termed secondary vacuoles. In histologic sections the secondary vacuoles were seen to correspond to the pyknotic nuclei. Further study showed that in nonirradiated suspensions incubated at 37°C., primary and secondary vacuoles also developed but at a slower rate than in irradiated suspensions. It was concluded that x-rays did not initiate any new degenerative process but accelerated the normal ageing, degeneration, and death of the lymphocyte.

These observations on the action of x-rays were made on thymic cells which were irradiated *in vitro* and were incubated aerobically at 37°C. The present study considers the question whether similar phenomena occur in thymic tissue irradiated *in vivo*.

METHODS

Anesthetized rats and rabbits were treated with 1000 r. units of x-ray irradiation over the thymic glands and the animals were sacrificed 1 to 6 hours later. One lobe of the gland was fixed in Bouin's solution and other fixatives. Paraffin sections of the tissue were stained with hematoxylin and eosin and with Feulgen's stain.

The other lobe of the irradiated gland was chopped up with scissors and filtered through a fine metal screen. To a small sample of the resulting suspension was added eosin in Tyrode's solution and a count was made of the number of stained (or dead) cells, unstained (or

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viable) cells, and red blood cells. By this method of unstained cell counts, it was possible to estimate the percentage of viable cells in the suspension. The suspension was examined also by dark-field illumination to determine the presence of primary and secondary vacuoles. Further details of the methods used have been presented in previous papers.[1-4]

Irradiated Thymic Glands

An unstained cell count of the suspension of a thymus, 3 hours after irradiation, showed that 85 per cent of the cells were viable (Fig. 1). In a control suspension of a normal thymus, 86 per cent of the cells were viable. Evidently, the technical procedures killed approximately 15 per cent of the cells in the two suspensions. Apparently the lymphocytes were not killed by the x-rays 3 hours after irradiation.

On dark-field examination of the irradiated cells, 35 per cent of the lymphocytes had primary vacuoles which appeared as single or multiple, dark, round structures usually surrounded by a vacuolar wall (Fig. 2). Some of the cells with multiple vacuoles were irregular. The vacuolated cells were similar in all respects to those seen in suspensions irradiated and incubated *in vitro*. In contrast, the suspensions from normal thymic glands had only an occasional vacuolated cell. The normal cells were round and were filled with fine intranuclear granules and had a few larger, brighter mitochondria (Fig. 3).

In the sections stained by Feulgen's method (Fig. 4), many nuclei had a round, central vacuole which either was unstained by Feulgen's method or took a light homogeneous stain. An occasional cell had two such clear areas. Surrounding the vacuole was a basophilic mass in the shape of a ring, horseshoe, or crescent. This mass usually stained deeply and uniformly, but in some cells it was composed of numerous fine chromatin granules.

Sections stained with hematoxylin and by Giemsa's method also showed cells with vacuolated nuclei. There appeared to be, however, fewer vacuolated nuclei in sections stained with hematoxylin than in those prepared by Feulgen's method.

On correlating the appearance of the living and the fixed cells, it would appear that the vacuoles observed on dark-field study corresponded with the unstained central areas in the sections. The ring or horseshoe-shaped, Feulgen-positive mass surrounding the vacuoles in the fixed sections probably corresponded with the bright, thick vacuolar wall observed under dark-field examination.

Thymic tissue removed 5 hours after irradiation showed, in histologic sections, few normal nuclei in the cortex. Most of the cells had pyknotic nuclei and a few had intranuclear vacuoles. In addition there were also many fragmented nuclei which stained deeply.

A suspension derived from this 5-hour thymus was prepared simultaneously. Examination of the eosin-stained cellular suspension showed numerous small, protoplasmic masses which varied in size down to 1 μ in diameter. The protoplasmic masses usually were unstained. They evidently corresponded to the fragmented nuclei seen in histologic sections. In spite of the failure of the small protoplasmic masses to stain with eosin, one had to assume that they were not viable. The failure of the protoplasmic masses to stain and the difficulty in differentiating a complete cell from a cell fragment made it difficult to obtain a satisfactory unstained cell count.

Dark-field examination of the suspension showed many cells with a single, large, secondary vacuole and a small crescent of fine and coarse granules. A thick, bright wall separated the vacuole from the surrounding media but there was no wall between the vacuole and the cytoplasmic granules. The fragmented nuclei seen in the sections and the unstained small protoplasmic masses observed in the hemacytometer were found, on dark-field illumination, to have thin but well illuminated walls (Fig. 5). Most of these structures were dark, but some of them had a few small granules which did not show brownian movement.

Normal Thymic Glands

According to the unstained cell counts, suspensions of untreated thymic tissue contained cells of which 14 per cent were dead and stained readily with eosin. It is believed that most of the dead cells were killed in the course of preparing the suspension. On dark-field examination a rare cell had a primary or secondary vacuole. In histologic sections an occasional cell with an intranuclear vacuole was found (Fig. 6). Evidently the degeneration of cortical cells by means of intranuclear vacuoles occurred *in vivo* in normal, nonirradiated thymic glands.

Discussion

Intranuclear Vacuoles

These studies by dark-field illumination showed that the first histologic reaction of lymphocytes to irradiation was the formation of one or a few small primary vacuoles within the nucleus. The formation and growth of the vacuoles were associated with an observed increase in the size of the cell.

Vacuolization and increase in the size of nuclei following irradiation probably have been observed by many pathologists in routine surgical and post-mortem tissue sections. As early as 1907, Warthin [5] described vacuolation of the nuclei of renal epithelium following irradiation. A good description of the phenomenon was given by Eckert and Cooper.[6] They observed that normal and malignant epithelial cells

of the uterine cervix underwent, after irradiation, three changes in the nuclei, namely, pyknosis, enlargement, and vacuolization. They found that the least common aberration in normal cells was vacuolization. The vacuoles varied in size up to the point of occupying the entire nucleus. They also varied in position and were either central or eccentric. Those located eccentrically were found usually in elongated nuclei and frequently were large enough to distend and distort one end of the nucleus. Occasionally, two or three vacuoles appeared in a single nucleus. In 3 cases of carcinoma of the cervix, the most notable change following irradiation was the presence of many vacuolated nuclei. The neoplasms of these cases received relatively small amounts of roentgen irradiation and all showed little effect from the therapy other than the increased vacuolation. The Feulgen stain was found to reveal more nuclear damage than the hematoxylin and eosin stain. Eckert and Cooper stated they could not explain the vacuolation of the nuclei, but suggested that the vacuoles were forms of localized degeneration of the nuclei.

Intranuclear vacuolization following irradiation has not, however, been described by some investigators. Akaiwa and Takeshima,[7] for example, irradiated the popliteal lymph nodes of rabbits. They found that during the first 12 hours the lymph node became enlarged and many cells showed nuclear disintegration. Intranuclear vacuoles were not observed although one would expect them, according to the present studies. The failure to observe post-irradiation, intranuclear vacuoles may be attributed to several factors. In the first place, these vacuoles are transient and the time of examination should be suitable for their demonstration. Secondly, the usual methods of fixation cause more or less shrinkage of cells and are not satisfactory for the preservation of intranuclear vacuoles. Finally, the predominating lesion in the irradiated tissue is the end-result of pyknosis and fragmentation, rather than the intermediate stage of vacuolization. As a result of these factors, investigators have overlooked or failed to describe intranuclear vacuolation. It is only when dark-field methods are employed that one realizes the high incidence of these vacuoles in irradiated cells.

Intranuclear Inclusions

To some extent, intranuclear vacuoles resemble intranuclear inclusions. A satisfactory definition differentiating between vacuoles and inclusions cannot at present be given, since the nature of these two structures is not known.

Intranuclear vacuoles have been observed in normal tissue and in tumors, according to the work of Roskin,[8] Osgood,[9] Apitz,[10] and others.

Similarly, intranuclear inclusions have been described in normal and malignant tissue. Bland and Russell [11] found intranuclear inclusions in tissue cultures of human meningiomata. They were visible in the living nucleus and had the appearance of vacuoles which closely resembled cytoplasmic vacuoles. They were optically empty by transmitted light but appeared granular by ultraviolet light. They could be seen by means of the dark-ground method. These authors, however, did not describe the appearance of the inclusions under dark-field illumination. In cultures stained with hematoxylin, the inclusions appeared as vacuoles limited by a membrane. Their internal structure was slight and consisted of a fine granulation or a spider's web of thin threads which stained pink with eosin. It would seem from this description that the intranuclear structures might be called vacuoles just as aptly, or perhaps even more suitably, than inclusions.

Acidophilic intranuclear inclusion bodies in human gliomas were reported by Russell.[12] Similar structures were found by Wolf and Orton [13] in glioblastomas and in meningeal and perineural fibroblastomas. Ludford [14] observed inclusion bodies with and without melanin in tissue cultures of human melanomas.

Fischmann and Russell [15] described inclusions in tissue cultures of human leptomeningioma, of rat and chick embryos, and of fibroblasts from the lungs of human and rat fetuses. Attempts to influence the development of the inclusions were unsuccessful. These authors also observed deep indentations or bays within the nuclear membrane so that the nucleus appeared as an incomplete ring. Similar structures were observed in the present study and described as horseshoe-shaped masses of chromatin material. Fischmann and Russell considered the possibility that the incomplete-ring nucleus closed and nipped off a cytoplasmic mass which became the intranuclear inclusion. In this study, it seemed that the ring nucleus ruptured at one point and ultimately discharged the contents of the vacuole into the cytoplasm. The present findings indicated that the ring nucleus preceded the horseshoe-shaped nucleus, and not the reverse as suggested by Fischmann and Russell.

Lucké [16] described prominent intranuclear inclusions in a tumor of the kidney of the frog, *Rana pipiens*. Covell [17] observed inclusions in tissues of apparently healthy monkeys. Both believed that the inclusions indicated the presence of a virus.

In view of the present studies, one might consider the possibility that the inclusions occurring spontaneously in normal and malignant cells were, like intranuclear vacuoles, signs of spontaneous degenerative changes in the cells.

Intranuclear inclusions or vacuoles have been produced by the use of various injurious agents, such as distilled water intravenously and salyrgan intramuscularly (Lee [18]), bismuth preparations (Pappenheimer and Maechling [19]), lead (Blackman [20]), aluminum and ferric compounds (Olitsky and Harford [21]), and burns (Belt [22]). Some of the structures produced by these agents had the typical features of intranuclear inclusions, namely, deep acidophilic staining, halo formation, and margination of chromatin. The inclusion bodies produced by some of these agents may be related to the inclusions that arise spontaneously in cells and to the vacuoles that are induced by x-rays.

The type form of intranuclear inclusions is that which occurs in virus diseases. The chemical attributes and the pathogenesis of these structures are not known. Cowdry and Kitchen [23] observed that in livers affected by yellow fever, many cells had empty and vesiculated nuclei. The empty nuclei were greatly enlarged, with clear, structureless centers, and appeared to be somewhat similar to those induced by hypotonic solutions in a previous study.[3] The vesiculated nuclei were not enlarged and had one or more vesicles of small and moderate size. The published descriptions of the vesiculated nuclei are similar to the vacuolated nuclei observed in irradiated thymic tissues in the present study. Intranuclear inclusions, empty nuclei, and vesiculated nuclei occurred together in the livers of patients with yellow fever. The coexistence of these three nuclear changes suggests a possible relationship in the pathogenesis of virus-induced inclusions and the vacuoles produced by spontaneous and induced degenerative changes.

It would appear that early workers sometimes failed to differentiate between intranuclear vacuoles and inclusions. More recently, Cowdry [23] and others have provided criteria for recognizing inclusions. The question remains whether there is any relationship between the two forms of nuclear degeneration. A study of inclusions and vacuoles by means of dark-field illumination may aid in demonstrating differences or similarities.

Degenerative Changes in Irradiated Lymphocytes

It is well known that the end-results of irradiation of lymphoid tissue are pyknosis, karyorrhexis, and karyolysis. The present work showed that an early or intermediate stage is the intranuclear vacuole. The course of events in the degeneration of a normal nucleus may be surmised from the dark-field observations and from examination of sections of an irradiated thymic gland. The hypothecated stages in the changes of the irradiated lymphocytic nucleus are shown in Figure 7. This figure was drawn from sections of a thymic gland of a rat

excised 3 hours after irradiation of 1000 r. Apparently the first manifestation of a change in the irradiated cell (Fig. 7, B) was a decrease in the staining of the central part of the nucleus and a shifting of the chromatin granules towards the periphery. At this stage, the nucleus had a small, lightly stained central zone and a thick ring of discrete chromatin granules. Later (D), there was an increase in the size of the central area and a decrease in the thickness of the ring, which now stained deeply and uniformly. The formation and growth of the intranuclear vacuole were probably associated with an enlargement of the nucleus. The continued increase in the size of the vacuole finally ruptured the ring of basophilic material, with the formation of a horseshoe-shaped mass (E and F). The outline of the nucleus could still be made out because of the presence of a thin, lightly stained nuclear wall. The contraction of the horseshoe-shaped mass produced first a crescent (G) and finally the small spherical pyknotic nucleus (H).

Summary

Intranuclear, acidophilic vacuoles were observed in many cells of thymic tissue of the rat and rabbit, 3 hours after irradiation with 1000 r. of x-rays. On dark-field examination of the suspension of the irradiated thymic tissue, many of the cells had single or multiple primary vacuoles with or without vacuolar walls. Thymic tissue, 5 hours after irradiation, had, on histologic examination, many pyknotic and fragmented nuclei. Suspensions of this tissue had many cells with secondary vacuoles and many fragments of cells. Nonirradiated thymus also had a few cells with intranuclear vacuoles and pyknotic nuclei. The spontaneous and x-ray-induced degeneration of lymphocytes was associated with the formation of intranuclear vacuoles, pyknosis, and fragmentation. Primary intranuclear vacuoles are seen in a higher percentage of cells by dark-field methods than by routine histologic technic.

Addendum

Two recent publications have described nuclear structures which may be similar to the intranuclear vacuoles reported in this paper. Rachmilewitz and co-workers [24] studied the effect of irradiation on bone marrow in tissue culture. The first sign of nuclear damage in erythroblasts and normoblasts appeared to be the accumulation of the chromatin at the periphery of the nucleus with a formation of a clear central zone. The nucleus was thus transformed into a large, almost colorless, sharply outlined vesicle. Dustin [25] made a detailed cytologic study of cells treated with various mitotic poisons. In intestinal epithelial cells of animals treated with hydroquinone, the degeneration of the nuclei resembled pyknosis in lymphoid tissue. The thymonucleic acid was found to accumulate on the nuclear membrane with the formation of a sphere composed of acidophilic protein and ribonucleoprotein. It is not definite from these publications whether the degenerative process with formation of a vesicle or sphere begins as a focal or diffuse process. Dark-field

studies suggest that the earliest change is a focal involvement with the formation of a small vacuole. This rapidly enlarges and involves the entire nucleus.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 172

FIG. 1. A mixture of an eosin solution and a suspension of a rat thymus removed 3 hours after irradiation (1000 r.) *in vivo*. The mixture is in a hemacytometer. Most of the cells are unstained, have fine granules, and are presumably viable. The stained cells are larger than the unstained, take a uniformly deep stain, and are presumably dead. × 550.

FIG. 2. Dark-field view of cells in a suspension of a rat thymus removed 3 hours after irradiation with 1000 r. *in vivo*. One cell in the upper photomicrograph and three cells in the lower have primary vacuoles which appear as large, round structures with or without a thin vacuolar wall. × 1250.

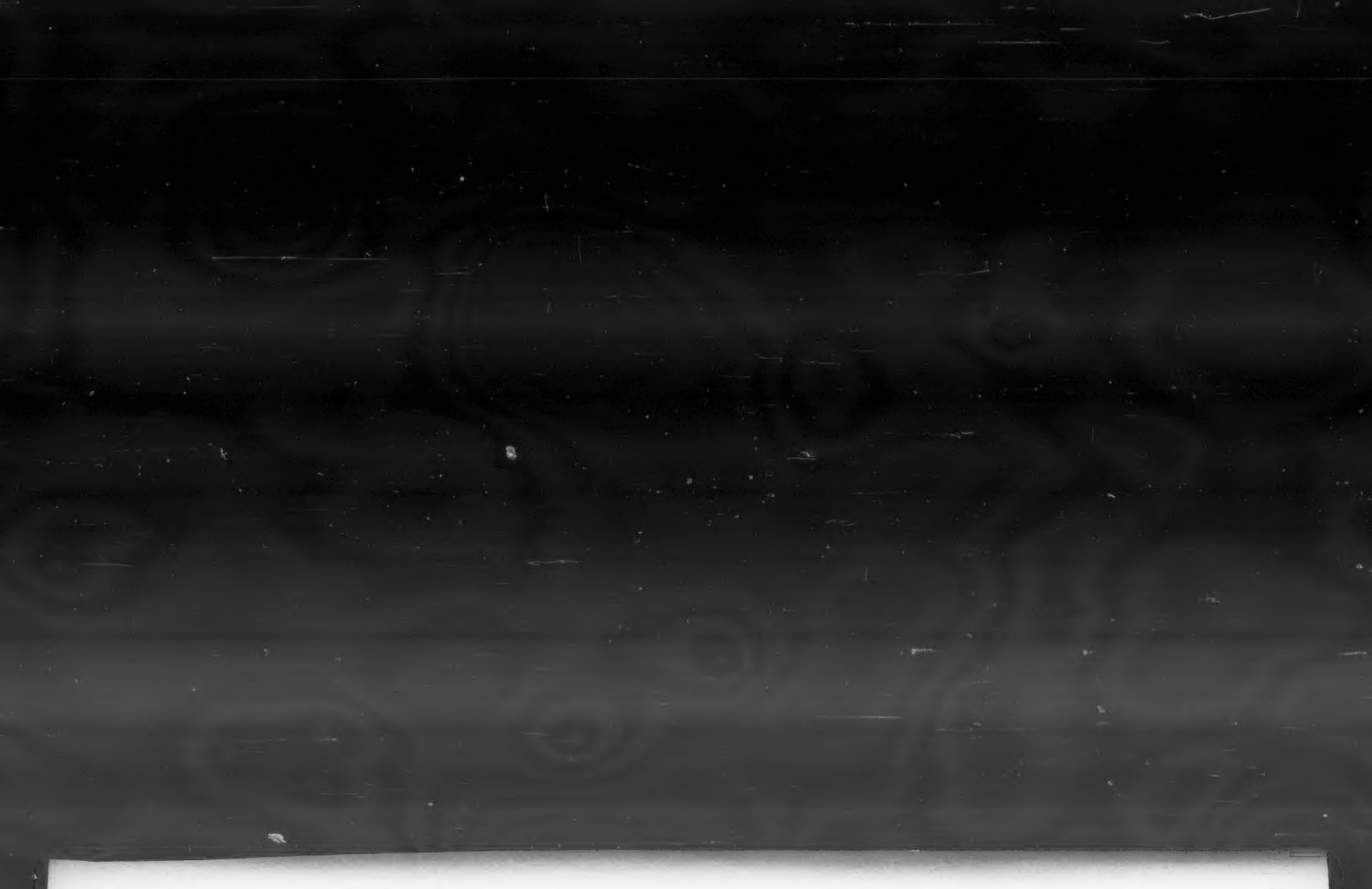
FIG. 3. Dark-field view of cells in a suspension of a normal rat thymus. The cells are round and filled with fine intranuclear granules. In one cell in the lower picture, the nuclear wall can be seen clearly. × 1250.

FIG. 4. Histologic section of a rabbit thymus excised 3 hours after irradiation *in vivo*. A few nuclei have small or large, central, unstained vacuoles surrounded by a ring or horseshoe-shaped mass of dense chromatin material. The section is stained by Feulgen's method. × 710.

FIG. 5. Dark-field view of cells in a suspension of a rat thymus removed 5 hours after irradiation (1000 r.) *in vivo*. The cell on the right side of the lower field has a large, eccentric, secondary vacuole and a mass of bright granules. The upper field shows a group of small, round masses with prominent walls and dark centers. These masses presumably represent fragments of nuclei which developed *in vivo* and are the result of irradiation. One cell in the lower area has three small, primary vacuoles. × 1250.

FIG. 6. Sections of the thymus from a normal rabbit. One cell in the upper picture and two cells in the lower picture have large, unstained vacuoles surrounded by horseshoe-shaped masses of dense chromatin material. × 1250.

FIG. 7. Drawings of nuclei found in a section of a rat thymus removed 3 hours after irradiation. The nuclei are arranged to represent the assumed course of events in the transformation of the normal nucleus to a pyknotic one. (A) Normal nucleus with fine granules. (B) Nucleus with small, central vacuole surrounded by a thick ring of finely granular, nuclear material. (C) and (D) Nuclei with large vacuoles surrounded by a thin ring of dense, structureless, basophilic material. (E) Rupture of basophilic ring with the formation of a large intranuclear vacuole surrounded by a horseshoe-shaped mass of basophilic material. The nuclear wall persists between the legs of the horseshoe-shaped mass. (F) and (G) Contraction of the chromatin material with the formation of a crescentic basophilic mass. The nuclear wall still persists. (H) Pyknotic nucleus with dense homogenous basophilic material.



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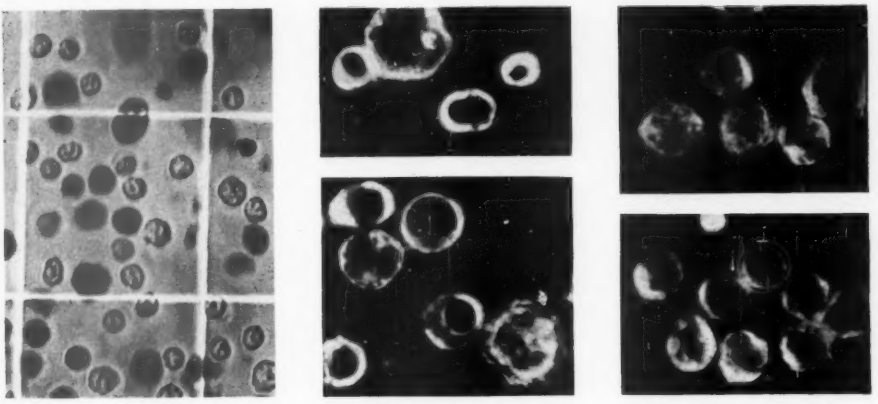

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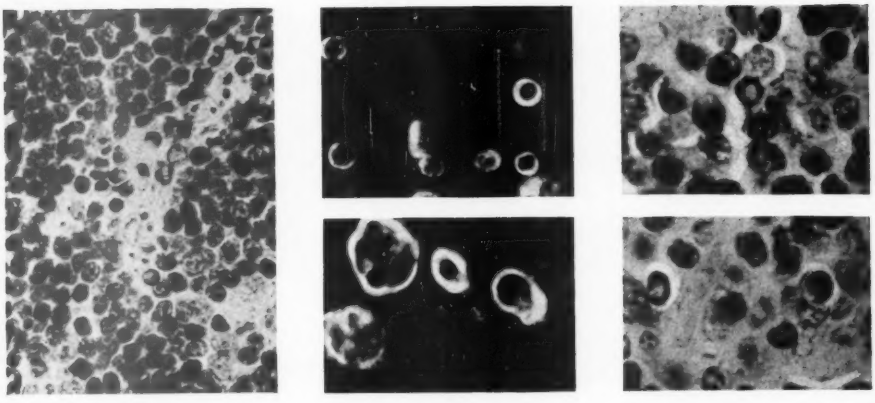

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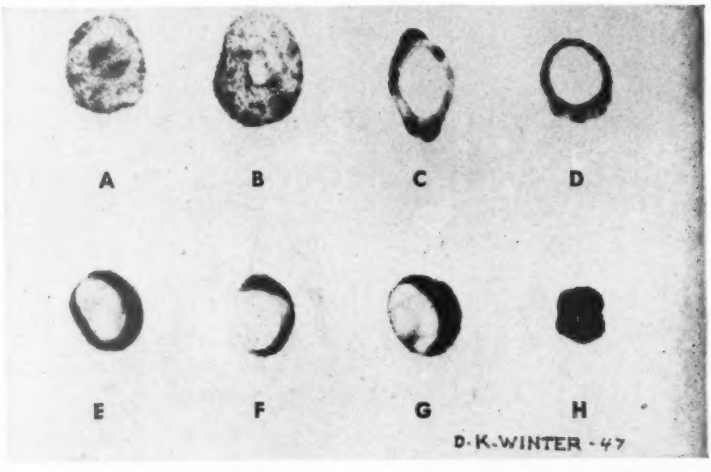

BASIC PATTERNS IN TERATOID TUMORS OF THE TESTIS *

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A series of 125 tumors of the testis was studied. Teratoid tumors comprised over 96 per cent (121 tumors) of these cases; the remainder consisted of one interstitial cell tumor, one mesothelial sarcoma of the tunicae testis, and 2 unclassified tumors. The teratoid tumors were analyzed according to basic histologic patterns. An attempt was made to correlate these patterns with the age incidence, hormonal activity, and mortality.

Included in the teratoid tumors are those commonly designated as seminomas, teratomas, embryonal adenocarcinomas, embryonal carcinomas, and chorio-epitheliomas.

HISTOLOGIC PATTERNS

Numerous preliminary surveys of all of the teratoid tumors revealed only three fundamentally distinct patterns. These were seminoma, teratoma, and carcinoma. Although deviations occurred in some of the tumors, these were considered to be of minor significance.

Seminoma Pattern

The tumor cells in the seminoma pattern were round or polyhedral and moderately large, although somewhat variable in size. The cytoplasm was abundant, and pale or clear in most instances. The nuclei were quite large, rarely multiple, had prominent nuclear membranes, and showed chromatin filaments arranged in a crosshatch pattern. The chromatin filaments often were concentrated toward the center of the nucleus. Mitotic figures were moderately numerous. The tumor cells usually were arranged in compact masses separated by fibrous connective tissue septa. There was infiltration of the fibrous septa by lymphocytes, occasionally plasma cells, and rarely multinuclear giant cells of the foreign body type. The degree of infiltration by these cells varied greatly from tumor to tumor and no line of distinction could be drawn between "seminoma with lymphoid stroma" and "seminoma without lymphoid stroma." Adjacent to the tumor, the seminiferous tubules showed varying degrees of atrophy. In some instances the seminiferous tubules were filled with large cells with clear cytoplasm closely resembling those of the tumor.

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Tumors of this pattern were apt to replace extensively the testicular parenchyma as a single lobulated mass; occasionally there were small satellite nodules. These tumors showed little tendency to invade the epididymis. Areas of necrosis often were present, and were generally more extensive in the larger tumors.

Carcinoma Pattern

The carcinoma pattern includes the types which have been called "embryonal adenocarcinoma" and "chorio-epithelioma." There was a great tendency for the embryonal adenocarcinoma and chorio-epithelioma patterns to merge. This tendency, together with the similarity in hormonal activity, makes it seem very likely that these two variants are histogenetically identical and that both arise from the chorionic plate or its derivatives.

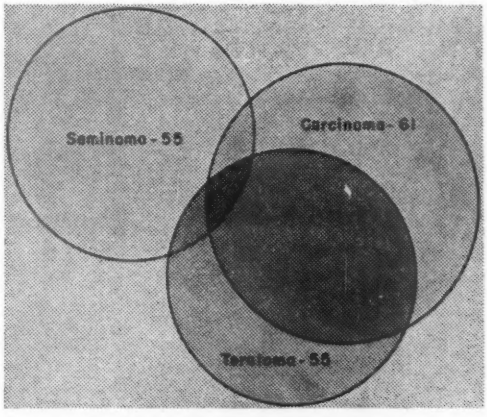
The embryonal adenocarcinoma variant was characterized by cells arranged in papillary or reticular formations, small compact groups, and perithelial clusters. Small areas of central necrosis were frequent. The tumor cells were quite variable in size but generally were larger than those in the seminoma pattern. The cell outlines usually were quite distinct. The cytoplasm varied from clear to eosinophilic and granular. The nuclei were large, with heavy nuclear membranes, and frequently were distorted and irregular; the chromatin often was clumped. A single, prominent, large, eosinophilic nucleolus was present. Mitotic figures were numerous. In many of these tumors there were focal areas showing large multinuclear giant cells (syncytial trophoblasts), in juxtaposition to smaller cells with hyperchromatic nuclei and pale cytoplasm (cytotrophoblasts). In some instances the multinuclear tumor cells were small and had pyknotic nuclei.

The chorio-epithelioma variant was characterized by masses of cytotrophoblasts and multinuclear giant syncytial cells. Occasionally, structures resembling placental villi were present. Active invasion and destruction of surrounding tissue were characteristic. Vascular invasion often was seen. Necrosis and hemorrhage occurred frequently in these tumors, and in many instances chorio-epitheliomatous elements surrounded the area of hemorrhage.

Teratoma Pattern

The teratoma pattern showed a wide range of variation from very immature embryonic structures to adult structures. At one extreme there were numerous, primitive organoid structures of epithelium and mesenchyme; at the opposite extreme there were easily recognizable, well differentiated structures such as intestinal mucosa, keratinizing

squamous epithelium, cartilage, and muscle. In some of the tumors of the teratoma pattern, structures of various developmental stages occurred together. In other tumors one element might tend to overgrow all others. Occasionally, the epithelial elements showed a tendency to dominate the picture, so that it was difficult to differentiate these areas from the carcinoma pattern. In still other tumors there were areas which appeared to be histologically malignant supporting tissue, *e.g.*, myosarcoma. No attempt was made to divide the tumors of teratoma pattern into those showing histologic malignancy and those showing histologic benignity. In our series no metastasis of only a *single* dif-



Text-Figure 1. Incidence and overlapping of basic patterns in teratoid tumors of the testis.

ferentiated tissue, such as muscle, bone, or skin, occurred; the metastatic lesions which contained tissues of the embryo proper showed the presence of differentiated tissue of more than one type.

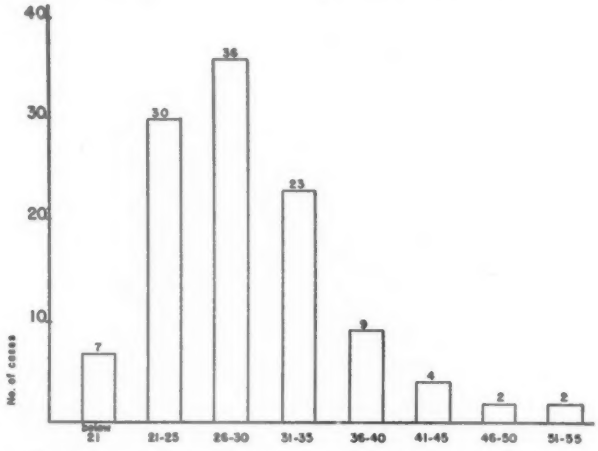
INCIDENCE AND OVERLAPPING OF BASIC PATTERNS

In the group of teratoid tumors of the testis, the three patterns of seminoma, teratoma, and carcinoma were encountered with approximately equal frequency. The seminoma pattern was encountered in 55, the teratoma pattern in 55, and the carcinoma pattern in 61 tumors. In 75 tumors a single pattern was present, and these tumors are those subsequently referred to as "pure." There were 44 pure seminomas, 13 pure teratomas, and 18 pure carcinomas. In 46 tumors various combinations of the three basic patterns were present (Text-Fig. 1). The teratoma-carcinoma combination was the one most frequently observed, and this was seen in 35 cases. The seminoma-teratoma com-

bination occurred in 3 cases, the seminoma-carcinoma combination occurred in 4 cases, and the seminoma-teratoma-carcinoma combination occurred in 4 cases. The confusing complexity of these tumors is due to a large extent to this tendency for more than one pattern to occur in the same tumor (*i.e.*, overlapping of patterns). It was felt desirable for the purposes of this study to designate a mixed or "impure" tumor by the particular patterns present.

AGE INCIDENCE

The ages of the patients ranged from 1½ to 53 years. The mean age for the entire group was 29.0 years. Over 80 per cent of these



Text-Figure 2. Age distribution of 113 teratoid tumors of the testis.

tumors occurred between the ages of 20 and 35 years. Text-Figure 2 shows the distribution of the teratoid tumors by age groups.

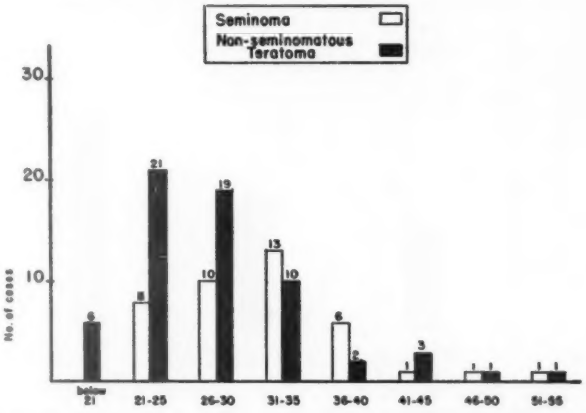
The age incidence of the tumors was studied with reference to the three basic patterns. The mean age of the pure seminomas was found to be slightly older than the mean age for all of the other teratoid tumors of the testis (Text-Fig. 3 and Table I). However, this difference was not found to be statistically significant. No significant differences could be demonstrated in the tumors of teratoma and carcinoma patterns.

HORMONAL ACTIVITY

The excretion of chorionic gonadotropin in the urine of many patients with teratoma testis is well known.[1-3] The production of interstitial cell hypertrophy and hyperplasia in laboratory animals by administration of chorionic gonadotropin is also well established.[4,5]

Hyperplasia of the interstitial cells of the testis has been noted also in many of our cases of teratoid tumor of the testis.

It was thought that it would be of interest to correlate the hyperplasia of interstitial cells with the basic tumor patterns of teratoid tumors. This phase of the study was limited to those cases in which there was sufficient testicular tissue remaining to permit an evaluation



Text-Figure 3. Distribution of pure seminoma and nonseminomatous teratoid tumors of the testis according to age.

of the status of the interstitial cells. The nature of the material did not allow a quantitative analysis. For this reason, emphasis was laid on the size of the individual interstitial cells and on the size of the groups of interstitial cells. We feel that the qualitative differences which were observed are significant because there was no correlation between the amount of testicular parenchyma replaced and the degree of interstitial cell hypertrophy and hyperplasia, indicating that this is not a compensatory hypertrophy.

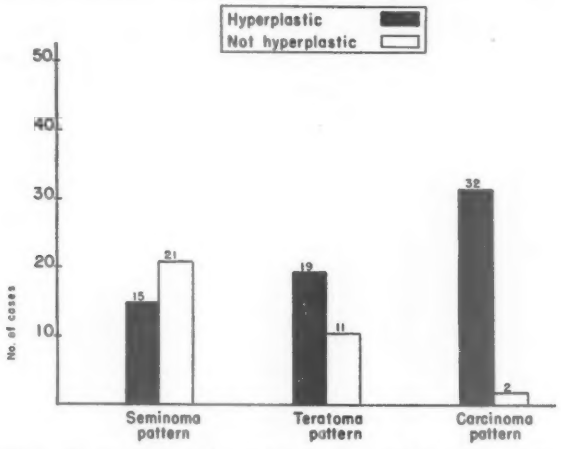
Of 72 tumors studied, there were 41 tumors (56.9 per cent) which were associated with interstitial cell hypertrophy and hyperplasia. The

TABLE I

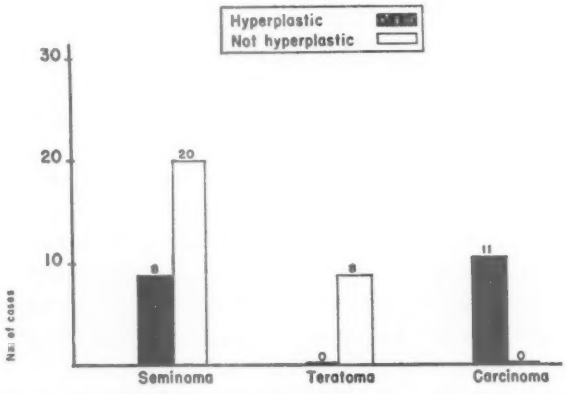
Statistical Comparison of Seminomas and Nonseminomas in Respect to Age of Patients

	No. of cases	Range	Mean
		years	*years*
Pure seminoma	40	21–53	31.35 ± 7.1
Teratoid tumors exclusive of those with seminoma element	63	1–53	27.74 ± 7.9

status of the interstitial cells was determined for each of the three basic patterns regardless of the presence of other patterns in the same tumor. The results of this analysis are presented in Text-Figure 4. Then the status of the interstitial cells was determined for each of the three



Text-Figure 4. Hyperplasia of interstitial cells in teratoid tumors according to pattern (including mixtures of impure tumors).



Text-Figure 5. Hyperplasia of interstitial cells in teratoid tumors with pure patterns.

basic patterns, excluding all cases which showed more than one pattern; in other words, only the pure tumors were included. The results are shown in Text-Figure 5.

The Friedman test [6] was performed on 42 cases prior to orchiectomy. A positive reaction was obtained in 13 cases (30.9 per cent), and a negative reaction in 29 cases (69.1 per cent). In 10 cases with a

negative Friedman reaction there was hypertrophy and hyperplasia of the interstitial cells. There was no instance in which there was a positive Friedman test and absence of stimulation of the interstitial tissue. Observations on the interstitial tissue and the Friedman reactions are summarized in Table II.

TABLE II

State of the Interstitial Tissue and Results of Friedman Tests in Respect to Basic Types

	Interstitial tissue		Friedman test	
	Hyperplastic	Not hyperplastic	Positive	Negative
Pure seminoma	8	20	0	12
Pure teratoma	0	8	0	1
Pure carcinoma	11	0	5	5
Seminoma-teratoma	1	1	0	2
Seminoma-carcinoma	3	0	0	1
Teratoma-carcinoma	15	2	8	7
Seminoma-teratoma-carcinoma	3	0	0	1

MORTALITY

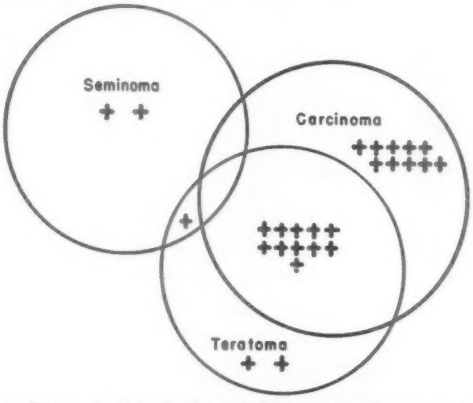
There have been 26 known deaths in this group. All but 2 have occurred within 2½ years after the tumor was discovered. In 3 cases the presenting symptoms were those due to metastases, and in one case the testicular tumor was found only at autopsy. In the 26 fatal cases the seminoma pattern occurred 3 times, the teratoma pattern 14 times, and the carcinoma pattern 21 times. The marked tendency of the teratoma and carcinoma patterns to occur in the same tumor again was noted, and in 11 of the fatal cases this combination was present. Only 2 deaths occurred in the pure seminoma group and 2 deaths in the pure teratoma group, whereas 10 deaths occurred in the pure carcinoma group. The results show that the carcinoma pattern has a much poorer prognosis (Text-Fig. 6).

There have been no deaths reported in our series in patients in whom interstitial cell hyperplasia was absent. There were 9 deaths in cases in which the available pathologic material did not allow an evaluation of the status of the interstitial tissue. Thus, on the basis of follow-up studies to date, absence of interstitial cell hyperplasia should be regarded as a favorable prognostic sign. On the other hand, 4 deaths have occurred in cases with a negative preoperative Friedman test.

DISCUSSION

The seminoma pattern showed relatively slight tendency to occur in conjunction with other patterns. On the other hand, the combination of teratoma and carcinoma patterns in the same tumor was encoun-

tered frequently. This latter finding supports the concept that the teratoma and carcinoma patterns are the result of differentiation of a pluripotent tumor cell into tissues of two types: those corresponding to the embryo proper (teratoma), and those corresponding to the derivatives of the chorionic plate (carcinoma). The occurrence of both teratomatous and carcinomatous elements in the same metastatic lesion suggests that the malignant cell is sufficiently undifferentiated and pluripotent to allow differentiation into these various elements after localization in the secondary site. We believe that the pure teratomas and pure carcinomas represent a unilateral development of a pluripotent cell.



Text-Figure 6. Distribution of deaths according to pattern.

In some instances within the teratoma group there was marked disorientation and proliferation of neurogenic, epithelial, or mesenchymal elements, suggesting malignant proliferation of only one element in a teratoma. However, no instance was found in which there was metastasis of only one type. Invariably, metastasis in such tumors consisted of teratoid tissue of more than one type, and usually included tissue showing the typical carcinoma pattern. For this reason, no attempt was made to designate a single element of a teratoma as being malignant, such as "rhabdomyosarcoma in a teratoma."

A close correlation between interstitial cell hyperplasia and the carcinoma pattern was noted; in *all* tumors with the carcinoma pattern in which the interstitial cells could be studied, hyperplasia was found. In no instance was absence of hyperplasia noted with a positive preoperative Friedman test. The remarkably close correlation of interstitial cell hypertrophy and hyperplasia with the carcinoma pattern, even in cases showing a negative Friedman reaction, would suggest that this finding may be used as an indication that tumor of the carci-

noma pattern is present. The occurrence of interstitial cell hypertrophy and hyperplasia in 28 per cent of the cases classed as pure seminoma suggests the presence of carcinoma patterns in these cases even though not demonstrated in the histologic sections. In 2 such cases examination of additional material resulted in the demonstration of carcinoma elements.

SUMMARY

In a series of 125 tumors of the testis, 96 per cent were found to be teratoid tumors.

Only three fundamentally distinct patterns, namely, seminoma, teratoma, and carcinoma, could be demonstrated consistently in the group of teratoid tumors. In 75 tumors only one pattern was present, whereas in 46 tumors there was more than one pattern.

The mean age for the entire group of patients with teratoid tumors was 29.0 years. The mean age for those with seminomas was 31.35 years; the mean age was 27.74 years for those with nonseminomatous tumors. This difference is not statistically significant.

Hypertrophy and hyperplasia of the interstitial cells of the testis were found to be very closely correlated with the carcinoma pattern.

In the known deaths which occurred in these cases, the carcinoma pattern was present much more frequently than the other two patterns.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

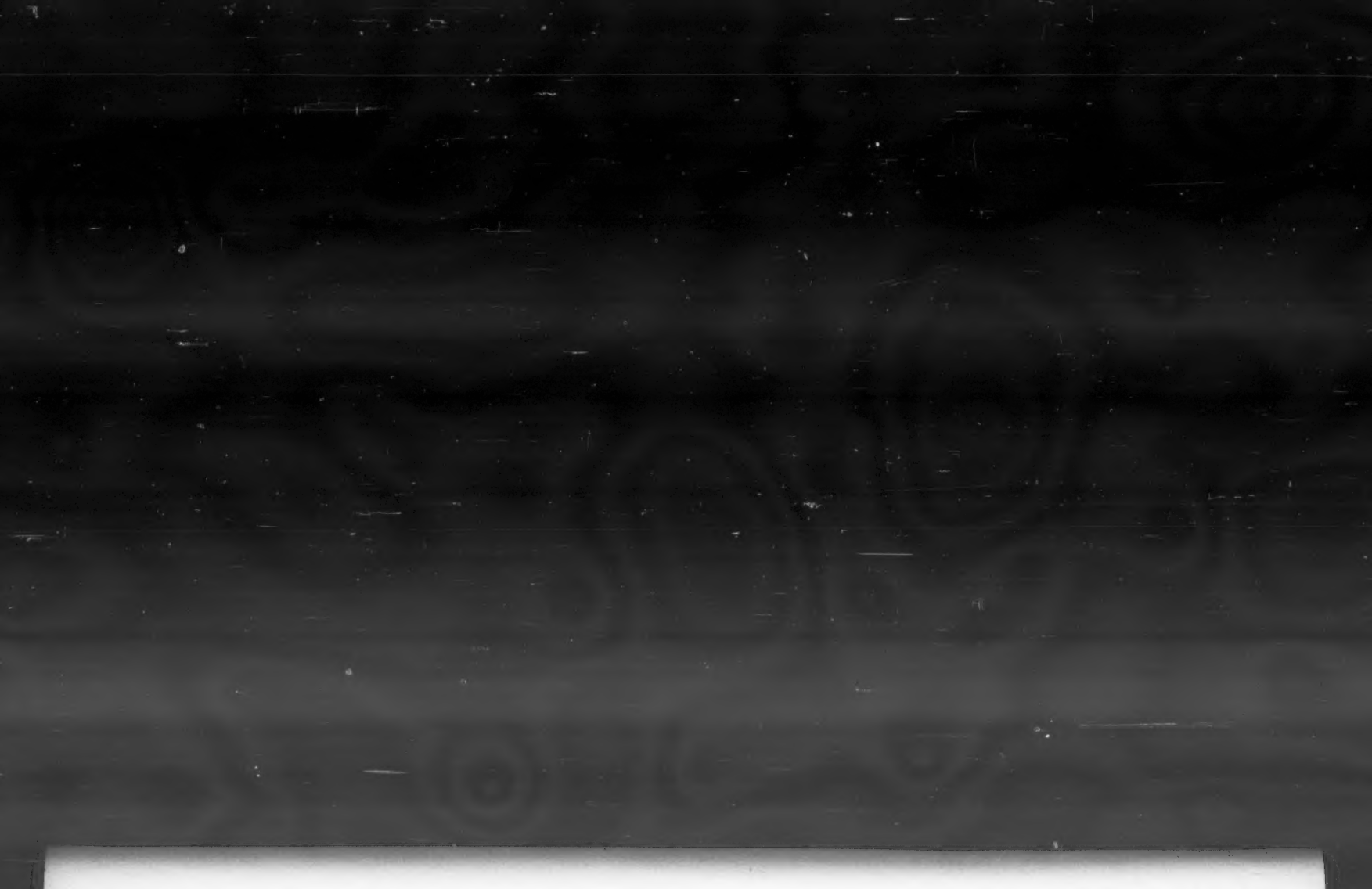
PLATE 173

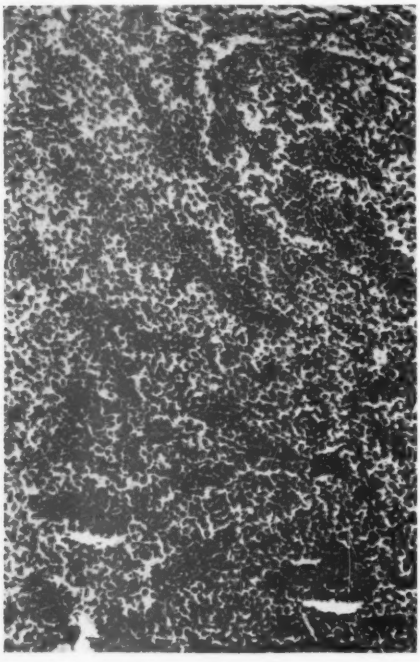
FIG. 1. Teratoid tumor of the testis of the seminoma pattern. × 50.

FIG. 2. Teratoid tumor of the testis of the seminoma pattern. × 100.

FIG. 3. Teratoid tumor of the testis of the seminoma pattern. × 50.

FIG. 4. Teratoid tumor of the testis of the seminoma pattern. × 100.





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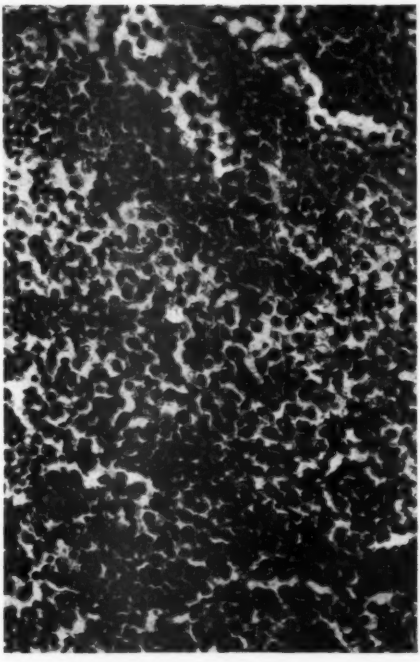

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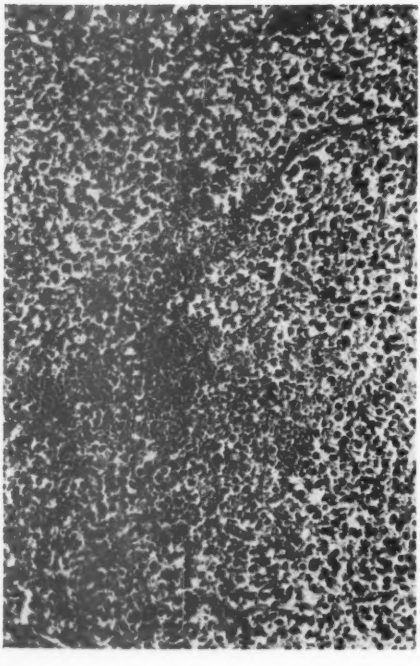

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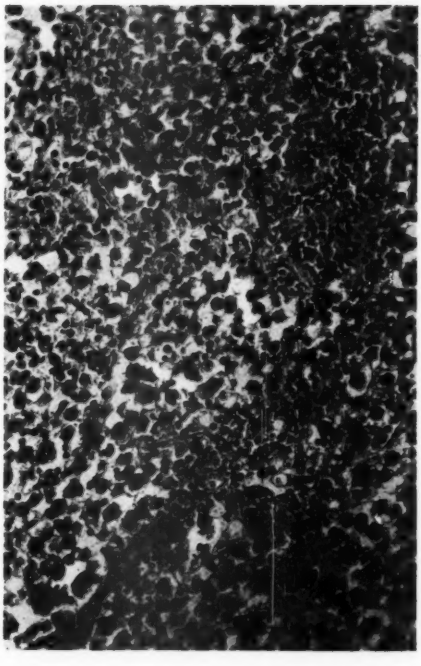

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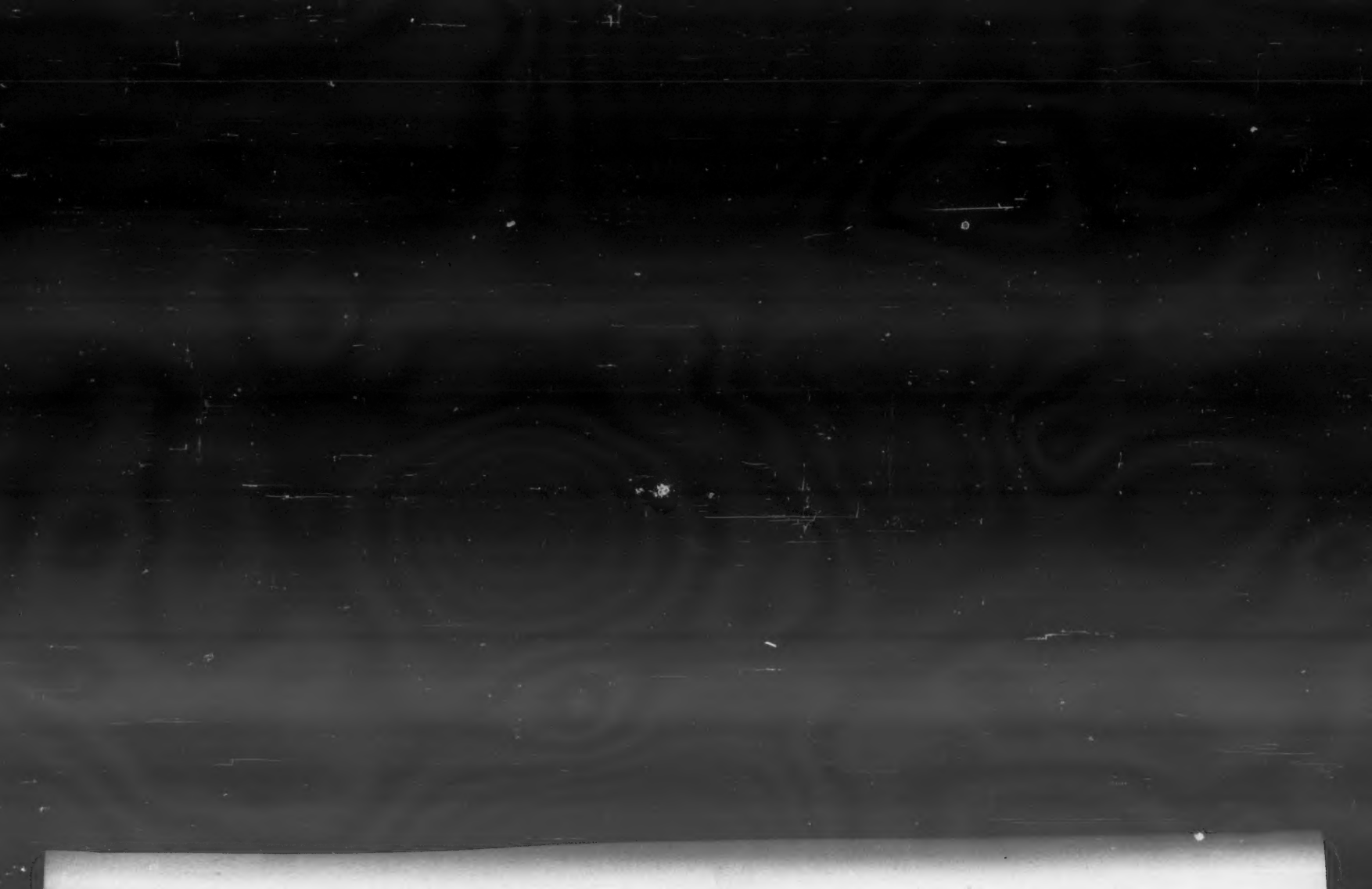
PLATE 174

FIG. 5. Teratoid tumor of the testis of the carcinoma pattern (adenocarcinoma variant). × 50.

FIG. 6. Teratoid tumor of the testis of the carcinoma pattern. × 50.

FIG. 7. Teratoid tumor of the testis of the carcinoma pattern (chorio-epithelioma variant). × 100.

FIG. 8. Hyperplasia of interstitial cells associated with a tumor of the carcinoma pattern. × 50.



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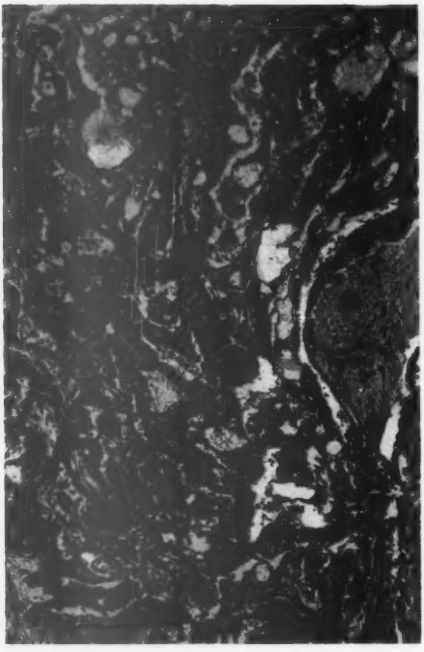

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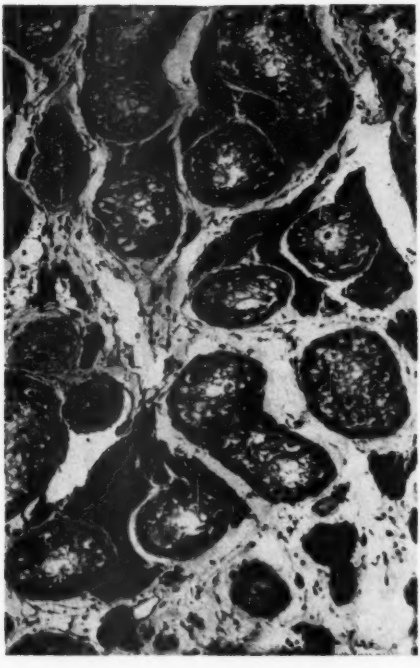


8

Moon and Hullinghorst Teratoid Tumors of the Testis

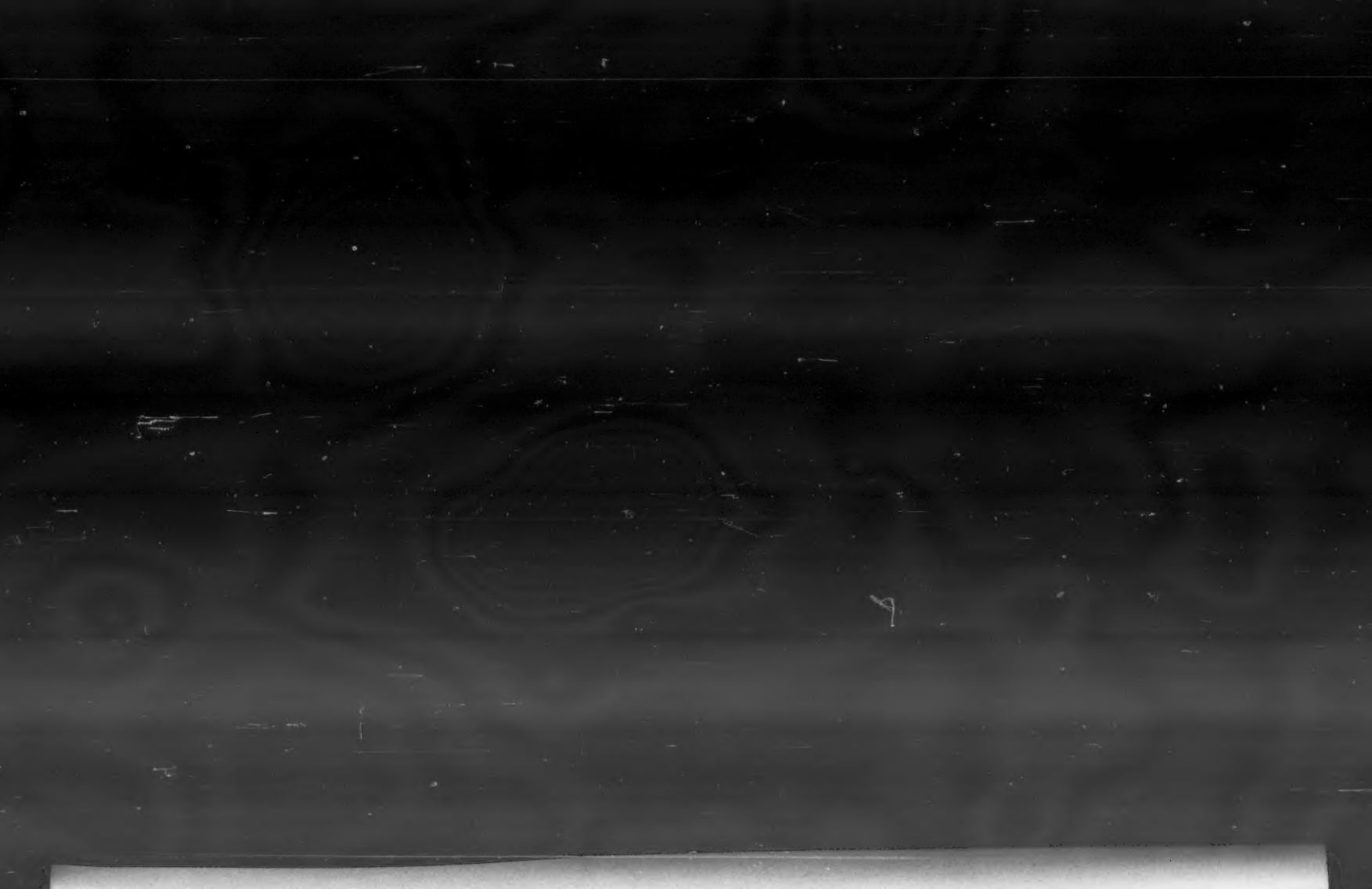
PLATE 175

FIG. 9. Testicular tumor of the teratoma pattern (embryonal variant). × 50.

FIG. 10. Testicular tumor of the teratoma pattern (adult variant). × 50.

FIG. 11. Combined seminoma and carcinoma patterns. × 100.

FIG. 12. Combined teratoma and carcinoma patterns. Both the adenocarcinoma and chorio-epithelioma variants are present. × 50.



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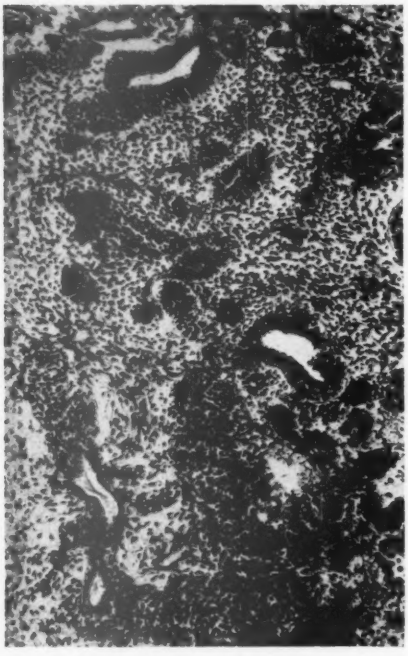

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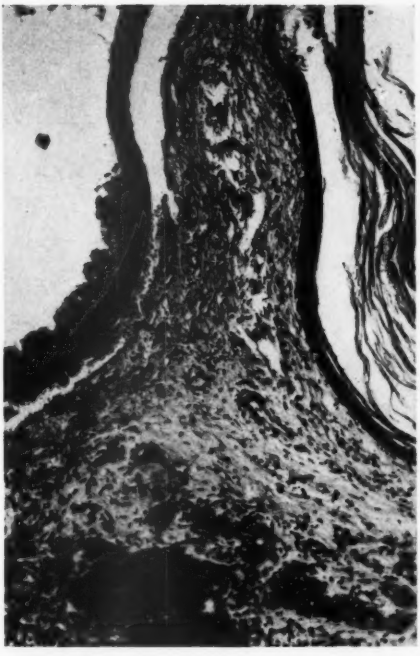

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PULMONARY ADENOMATOSIS
A Report of Three Cases *

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(From the Research Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Ill.)

Helly,[1] in 1907, described a multiple, nodular, bilateral tumor of the lungs in a woman, 43 years of age. Because of the preservation of the alveolar septa which were lined with cylindric cells and the absence of metastases, he termed the growth an adenoma, as distinguished from an alveolar cell carcinoma.

Of considerable current interest, pulmonary adenomatosis is not without curious and speculative features. Since Helly's report, 12 cases have been described in the literature [2-12]; 6 of these have been reported within the past several years. The renewed interest in the subject perhaps has been stimulated by Bonne's [2] observations on the morphologic resemblance of pulmonary adenomatosis in sheep (jaagsiekte) to certain cases of pulmonary cancer in man.

Epizootic pulmonary adenomatosis of sheep is of global distribution. In 1915 Mitchell [12] extensively described the condition, and in 1938 Dungal [13] reported an epidemic in Iceland. The usually fatal ovine disease, the lesions of which resemble pulmonary lesions found in sheep suffering from sheep-pox, consists of a certain amount of interstitial fibrosis and a tumor-like proliferation of pulmonary epithelium. It has in common with human pulmonary adenomatosis multicentricity, columnar nonciliated cells, absence of stromal invasion, and infrequent mitotic figures.[10] No specific agent has been proved to be the cause of the disease, but many investigators [2, 9, 14] have suspected that a virus is the inciting and responsible factor. Transmission of the disease is affected easily by housing healthy sheep with diseased sheep. However, experimental infection has been generally unsuccessful; in only one instance was the condition reproduced in a sheep by an intrapulmonary injection of a tissue suspension.[15] Efforts to isolate a virus from a human specimen of pulmonary adenomatosis were not successful.[8] On the other hand, Grumbach [16] found diffuse alveolar epithelization after inoculating a guinea-pig with a diphtheroid bacillus obtained from a lymph node of a patient with Hodgkin's disease. Subcutaneous injection of 1, 2, 5, 6-dibenzanthracene into mice produced multiple malignant tumors of alveolar origin.[17] Norris [18] injected fluid aspirated from a patient with lobar pneumonia into a guinea-pig. One lung of the guinea-pig subsequently contained lesions resembling those of jaag-

* Received for publication, October 20, 1947.

siekte in sheep. In various spontaneous pathologic conditions of the lung, epithelium-like lining cells are found along the alveolar walls,[7, 19] and it has been suggested that the difference between simple localized hyperplasia or metaplasia and adenomatosis may be more apparent than real.[8]

The evidence seems to indicate that pulmonary adenomatosis is a specific response to certain nonspecific irritants. This hyperplastic response eventually causes death by extensive involvement of lung parenchyma or by progressing into a true malignancy.

The gross pathology of pulmonary adenomatosis usually resembles pneumonia in gray hepatization. Occasionally mucus can be scraped from the cut surface. Nodular consolidated areas, which have been compared to noncaseating miliary tuberculosis, have been described. A primary bronchial focus has never been demonstrated. Nonciliated cuboidal or columnar cells, lining otherwise unaffected alveolar walls, characterize the microscopic changes. The cytoplasm of these cells is eosinophilic, granular, or somewhat foamy. Goblet formation and a brush border may be noted. The nuclei are usually basally or subcentrally located; nucleoli may be prominent and the chromatin is coarse. In general, the cells are uniform and mitotic figures are uncommon. The alveolar septa are not involved or show only slight thickening; absence of invasion is a feature of the disease. The alveolar spaces contain desquamated cells, occasional phagocytes, and a variable amount of exudate and mucus. Bronchopneumonia often obscures the significant features of the condition.

With microscopic features identical with those of the so-called alveolar cell carcinoma, pulmonary adenomatosis differs from carcinoma by virtue of its presumably nonmetastasizing nature. However, of the 12 previously reported cases of adenomatosis, 2 showed metastatic lesions.[4, 5] In one of the cases presented in this study malignant proliferation was noted (Fig. 6). Metastasis has occurred in jaagsiekte of sheep. There is no reason to assume that pulmonary adenomatosis is forever a benign growth, and it is possible that pneumonia, which usually complicates the disease, may cause death before metastasis can develop.[11] It is likely that adenomatosis of the lungs is a relatively benign variant of the so-called alveolar cell carcinoma and is potentially capable of developing all of the characteristics of malignancy. We feel relatively certain that a case of so-called alveolar cell carcinoma previously reported from this laboratory was actually a case of pulmonary adenomatosis.[20] Of academic interest and of great controversy is the cellular origin of these tumors. Theoretically, the cells may be derived from bronchiolar or alveolar epithelium. Loosli [21]

and others [22, 23] have maintained that no true alveolar lining exists and that the capillaries are contained in a ground substance with infrequent cells of mesenchymal origin. Other workers [24, 25] believed that the alveoli have a continuous epithelial lining. Because of the decided epithelial nature of the adenomatous cells and, more particularly, the absence of cilia, either the lining cells of the alveoli or the lining cells of the terminal bronchioles are the cells of origin of these tumors. Since the number of those who deny the existence of alveolar epithelium is increasing because of inability to demonstrate the cells,[26] the possibility of a bronchiolar origin probably should be given preferential consideration. In one of the cases presented in this report (Figs. 2 and 3), several terminal bronchioles with normal epithelium that abruptly shifted to columnar adenomatous cells were seen.

It is unfortunate that there are no signs or symptoms pathognomonic of pulmonary adenomatosis. Radiologically, the condition simulates tuberculosis, carcinoma, or pneumonia. The patients give no history of exposure to sheep. Dyspnea and a cough productive of mucoid sputum are prominent inaugural symptoms. Statistics on bronchogenic carcinoma show a ratio of 3 men to 1 woman; in the so-called alveolar cell carcinoma the ratio is 1:1. In the collected cases of pulmonary adenomatosis, including those reported here, there were 9 women and 6 men. Their average age was almost 54 years. The duration of the disease has varied from several weeks to over 2 years. The incidence of pulmonary adenomatosis in our series of 57 cases of lung carcinoma is 5 per cent. Neubuerger and Geever [26] also estimated the incidence of alveolar cell carcinoma as 5 per cent.

Report of Cases

Case 1

H. D., an unmarried woman of Polish ancestry, 34 years old, had lived in Chicago all of her life. She had been a hospital attendant for 10 years and there was some contact with tuberculous patients. Admitted to the Municipal Tuberculosis Sanitarium on July 1, 1946, her symptoms consisted of a nonproductive cough and a weight loss of 4 lb. A routine roentgenogram of the chest a few months previously had disclosed a 3 cm. cavity in the right lower lobe. Her general health was good.

On physical examination, abnormal findings were limited to the chest. There was slightly impaired expansion with harsh breath sounds in the right lower lobe anteriorly. The vital capacity was 2600 cc. There was no fever. A bronchogram revealed no abnormalities. The Mantoux test was positive, and all sputum examinations were negative for tubercle bacilli. Staphylococci were cultured from the sputum; there was no anaerobic growth. The red blood cell count was 4.2 million, the hemoglobin was 69 per cent. The white blood cell count was 4,900 with 46 per cent polymorphonuclear leukocytes, 39 per cent lymphocytes, and 14 per cent monocytes. Kahn test of the blood was negative, and the urinalysis was normal. Bronchoscopy was negative except for signs of chronic suppuration of the right lower lobe.

The patient was given 1 million units of penicillin. There was some clearing of the soft infiltrate about the cavity wall as determined roentgenologically. She was discharged on October 30, 1946, with a diagnosis of cavity of the right lower lobe of undetermined origin.

On January 9, 1947, a roentgenogram of the chest showed no changes in comparison with previous films.

On June 3, 1947, the patient was readmitted. During March and April she had suffered two attacks of bronchopneumonia with a fever up to 105° F., hemoptysis, and severe pain over the right lower chest. On examination there was increased vocal fremitus over the right lower lobe with dullness, bronchial breathing, and amphoric breath sounds posteriorly. Some consonating râles were heard in this area. A roentgenogram showed consolidation and atelectasis of the right lower lobe. Total blood protein was 8.5 gm., and the white blood cell count was 6,200 with 64 per cent polymorphonuclear leukocytes. Other laboratory findings were unchanged from those of the previous admission.

A lobectomy was performed by Dr. Richard Davison on June 19, 1947. The preoperative diagnosis was chronic lung abscess with pneumonitis and bronchiectasis. The postoperative course was uneventful.

On gross examination, the right lower lobe of the lung was firm throughout. There were a few thin, fibrous tags on the pleural surface. On section, the parenchyma was uniformly solid and resembled the gray hepatized stage of pneumonia. There was no mucous exudate. At the apex of the lobe was a small (2 cm.), irregular cavity with ragged walls and an absence of exudate. Microscopically, there were tall, thick, nonciliated columnar cells which lined the alveolar walls. The cytoplasm was eosinophilic and slightly granular. The nuclei were regular in size and basally situated. Mitotic figures were uncommon. Papillary formation was noted frequently and the alveoli contained a few desquamated columnar cells, occasional polymorphonuclear leukocytes, and a slight amount of mucus. In the area of ulceration there was marked fibrous hyperplasia with degeneration of the adenomatous cells. Elsewhere the alveolar septa were intact. A bronchiole showed transformation of the normal mucosa to tall-columnar nonciliated cells (Fig. 2). No tumor cells were evident in the pleura, the lymph spaces, or in the blood vessels. The bronchial lymph nodes were not dissected at the operation.

Case 2

N. D., a white woman, 55 years of age, developed a mild cough in August, 1939. Pulmonary tuberculosis was diagnosed by her private physician after roentgenologic examination of the chest. She entered the Municipal Tuberculosis Sanitarium in December, 1939, complaining of a cough and a small amount of sputum which was occasionally streaked by blood. Fatigue and weakness were noted.

On physical examination the patient was found to be in good general condition. The blood pressure was 160/100 mm. Hg. Expansion of the chest was limited and resonance was impaired throughout the right upper one-third and throughout the entire left hemithorax. Coarse breath sounds with scattered moist râles were heard in these areas. A roentgenogram revealed areas of marked haziness in both lungs, most pronounced in the right. The cardiac shadow was displaced to the left.

All sputum examinations were negative for tubercle bacilli. *Streptococcus*

viridans was cultured from the sputum. The hemoglobin was 85 per cent; white blood cell count, 9,250 with 66 per cent neutrophils. The sedimentation rate was 6 mm. in 30 minutes (Cutler). The Mantoux test was positive and Kahn test of the blood was negative. The urinalysis gave normal findings. A sputum section on January 14, 1940, showed many epithelial cells which were single or in small groups. These cells were ovoid with round, vesicular nuclei and eosinophilic cytoplasm. Some nuclei were hyperchromatic. The diagnosis was pulmonary carcinoma. An aspiration biopsy of the lung a few days later was diagnosed as squamous cell carcinoma.

The patient became progressively more dyspneic and weaker. Death occurred on February 16, 1940.

Autopsy was performed 9 hours after death; permission was granted to remove only the lungs. A thin deposit of fibrin covered each lung and there were multiple adhesions throughout. Moderately distended and slightly anthracotic, both lungs on section contained several firm, dull gray areas in the mid-zones. Round, rather sharply defined and occasionally confluent, these lesions were similar in appearance to gray pneumonic hepatization. In the right base a firm gray nodule was found beneath the pleura. Except for edema the remaining lung fields were uninvolved. A frothy fluid filled the bronchi. The hilar lymph nodes were of normal size and contained no tumor metastases.

Microscopically, the septa were lined by high, nonciliated columnar cells with abundant eosinophilic cytoplasm and an occasional brush border. The nuclei were oval, situated basally, uniform in size, and somewhat vesicular. Papillary formation was pronounced, but mitotic figures were infrequent. There was slight thickening of the alveolar septa. Disseminated throughout was an acute necrotizing bronchopneumonia with marked inflammatory exudation and infiltration. A fibrinous pleuritis was extensive. Attended by necrosis, organization and early fibrosis, the adenomatous cells in one small area had lost all attributes of differentiation (Fig. 6). Anaplastic, invasive, and destructive, the tumor cells were irregular in size and shape and mitotic figures were common. The anatomic diagnosis was pulmonary adenomatosis with malignant degeneration.

Case 3

C. Z., a white housewife, 45 years old, was admitted to the sanitarium on October 2, 1934. Two years previously she had suffered an attack of influenza of 3 weeks' duration which left her fatigued. After the discovery of tubercle bacilli in the sputum and roentgenologic evidence of a pulmonary lesion, pneumothorax was established in May, 1933. Because of progressively severe dyspnea, the pneumothorax was discontinued after 1 year. In August, 1934, she suffered a pulmonary hemorrhage of 10 oz. Since the onset of her illness there had been an intermittent cough which was usually nonproductive. Weight loss was 25 lb. Her appetite was poor and her strength was failing.

Physical examination revealed a woman in fair general condition. The fingers were clubbed. The blood pressure was 112/70 mm. Hg. Chest expansion was

impaired bilaterally and vocal fremitus was increased over the right base. Throughout the right chest and over the lower one-half of the left, resonance was impaired. Breath sounds were harsh in these areas. Posttussive râles were heard throughout the right lung. Roentgenograms showed marked hazy infiltration of the right lung and of the middle field of the left lung. Bronchoscopy and esophagoscopy revealed no abnormalities. The red cell count was 4.2 million with 70 per cent hemoglobin. The white blood cell count was 10,600 with 85 per cent polymorphonuclear leukocytes. Kahn test of the blood was negative. No malignant cells were found in a sputum section and repeated sputum examinations were negative for tubercle bacilli. The urinalysis was negative.

In spite of bed rest the dyspnea continued. The diagnosis was far advanced tuberculosis, although bronchogenic carcinoma was considered. The patient died on March 8, 1935.

Autopsy was performed 6 hours after death. The right pleural space was completely obliterated by fibrous adhesions which were most marked in the right upper lobe. The entire right lung was consolidated and on section was gray and hepatized. In the left lung a similar lesion fanned out from the hilum and reached the pleura laterally and the base posteriorly. The bronchi and pulmonary lymph nodes were normal. Other autopsy findings were not related to the pulmonary disease.

Microscopically, much of the lung tissue was replaced by areas of large, irregular, branched alveoli which were lined by cuboidal cells (Fig. 4). The nuclei of these cells were round, basophilic, and sometimes hyperchromatic. The cytoplasm was eosinophilic. While occasional columnar cells were seen, there was little papillary formation. Mitotic figures were rare. The interstitial tissue was much thickened by loose connective tissue and there was much proliferation of lymph vessels. There was no necrosis and the pleura was not infiltrated. An old encapsulated caseous lesion without signs of perifocal inflammation was found.

Summary

Three cases of pulmonary adenomatosis are presented in this report. Case 1 is the second recorded surgical specimen of this condition and indicates a possible bronchogenic origin for the disease. Case 2 illustrates malignant proliferation of the cells of the adenomatous process. The cuboidal epithelium lining the air sacs and the loss of normal pulmonary architecture in case 3 represent an unusual variety of adenomatosis of the lung.

Representing about 5 per cent of all pulmonary tumors, adenomatosis of the lungs resembles the so-called alveolar cell carcinoma in all respects.

Probably the result of nonspecific irritation, the hyperplastic reaction of pulmonary adenomatosis is aggressive and may develop all the characteristics of malignancy.

There are no distinguishing clinical features of pulmonary adenomatosis. The average age of the patient is about 54 years, and the ratio of women to men is 3:2. The course of the disease varies from several weeks to 2 years.

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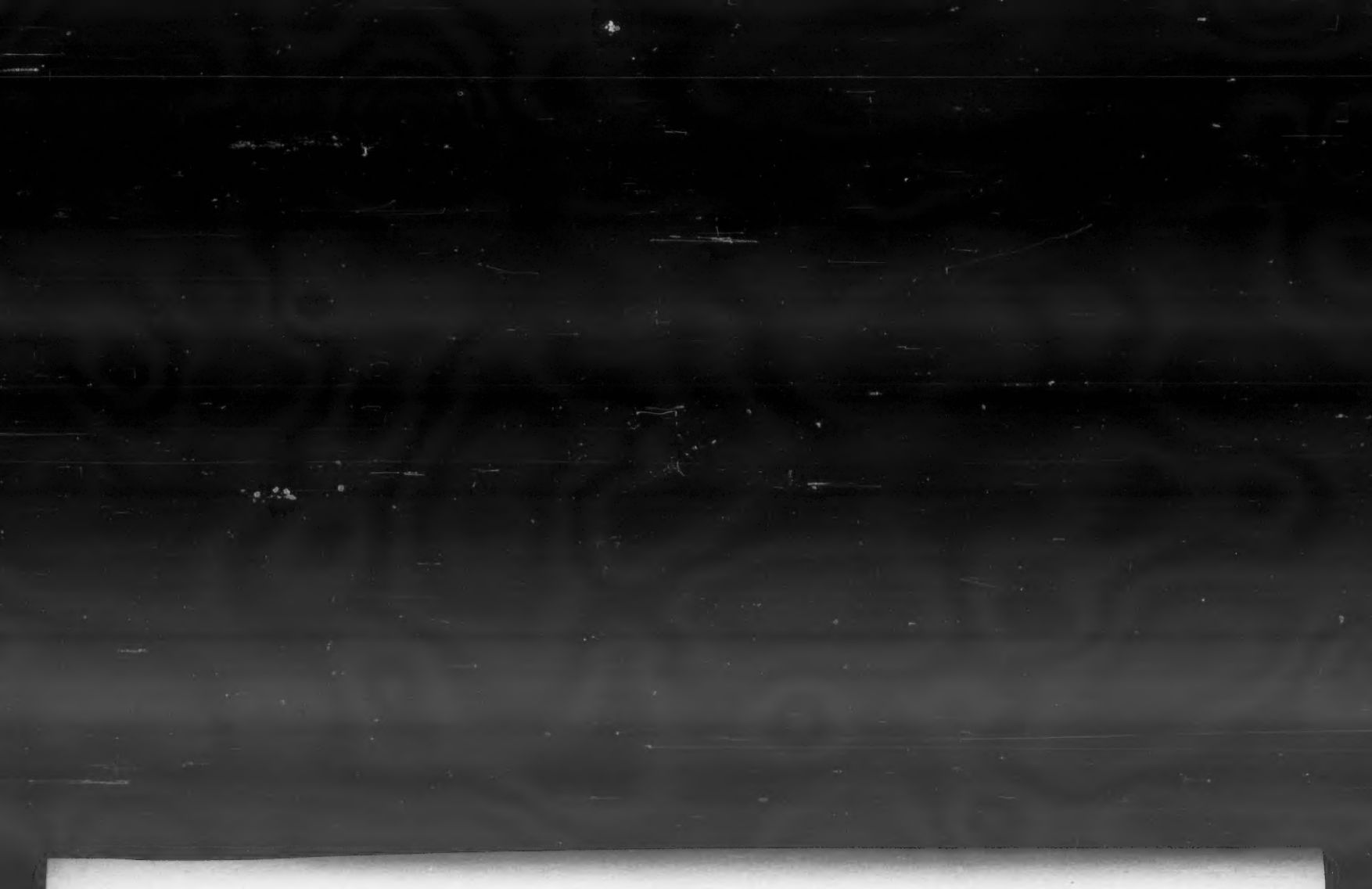
DESCRIPTION OF PLATES

PLATE 176

FIG. 1. Case 1. Photograph of the right lower lobe showing ragged ulceration and gray consolidation.

FIGS. 2 and 3. Case 1. Photomicrographs showing abrupt transformation of bronchiolar epithelium to nonciliated columnar cells and adenomatous cells lining the unaffected alveolar walls. × 300 and × 150.



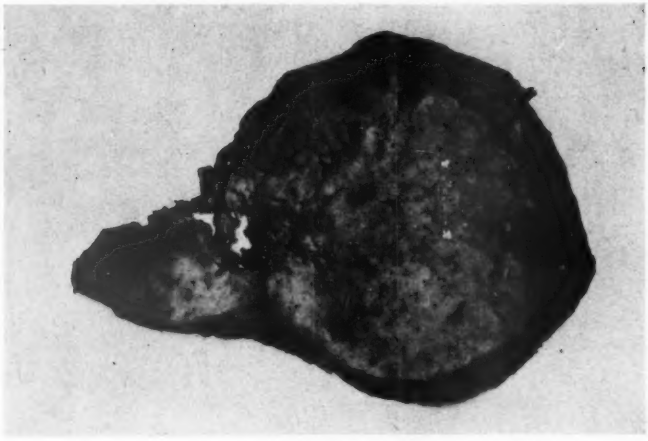


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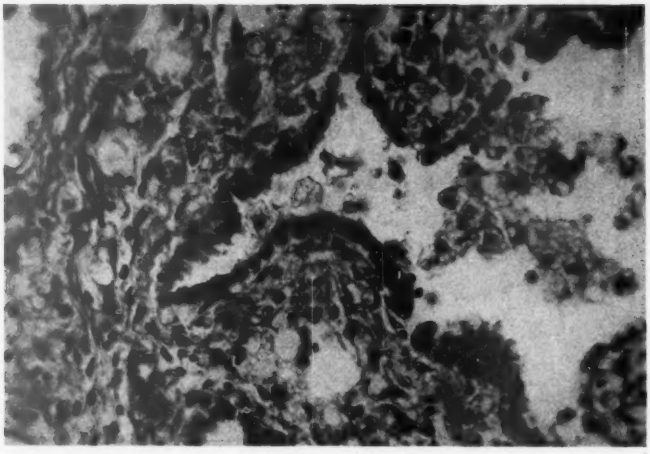
PLATE 177

FIG. 4. Case 3. Photomicrograph showing cuboidal cells lining the air spaces and interstitial fibrosis. × 300.

FIGS. 5 and 6. Case 2. Photomicrographs showing columnar cells together with pneumonic exudate and the focus of malignant proliferation found in the same lung. × 150 and × 300.



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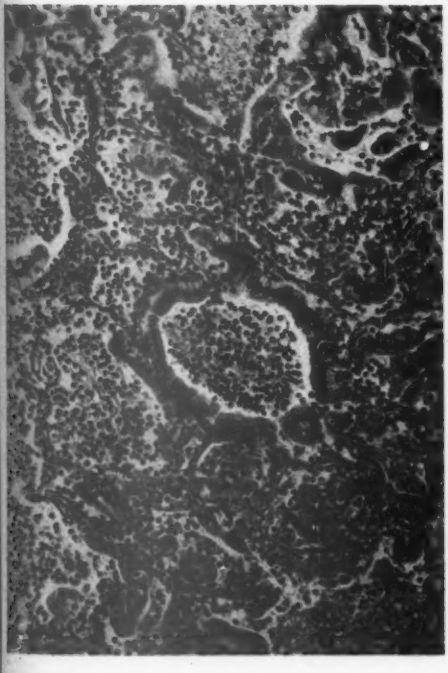

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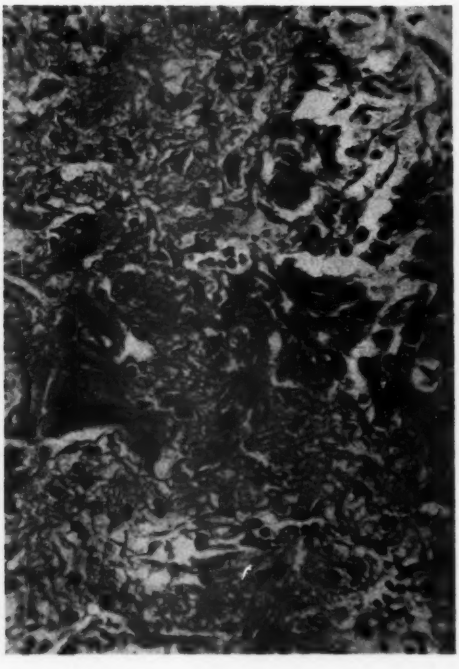

6

ATYPICAL AMYLOID DISEASE, WITH OBSERVATIONS ON A NEW SILVER STAIN FOR AMYLOID *

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There has been no general agreement on the classification of amyloid disease. Not only is there a bewildering array of names, such as primary, secondary, atypical, local, systemic, tumor-forming, and paramyloidosis, but there is no uniformity in regard to the meaning or scope of these terms. One of the most interesting manifestations of the disease has been described as primary amyloidosis, a term especially popular in the American literature, indicative of no known cause or pre-existing disease which might be held responsible for the amyloid deposition. The two most complete reviews of the subject are the papers of Koletsky and Stecher [1] and, more recently, of Lindsay.[2] Koletsky and Stecher listed 22 cases, as of 1939, while Lindsay in 1946 found 45. Several other reports are mentioned by Lindsay but not considered acceptable by him. In the absence of satisfactory criteria, it may be stated that approximately 50 cases are described in the literature.

The purpose of the present communication is threefold: To present 6 cases of unusual amyloid disease, 5 of which appear similar to the rare primary form; to describe a new silver stain for amyloid; and to analyze the confusing nomenclature and terminology, and suggest a new classification.

REPORT OF CASES

(Case 1 is from the Fairfield State Hospital, Newtown, Connecticut, obtained through the courtesy of Dr. W. F. Green, Superintendent. The other 5 cases are from the Illinois Masonic Hospital, Chicago, Illinois.)

Case 1

The patient was a white male, 88 years old. He was a pauper, brought to the Fairfield State Hospital showing marked mental deterioration in all spheres. The heart sounds were of poor quality, and severe peripheral vascular sclerosis was present. The blood pressure was 140/70 mm. Hg. Moderate tremor of hands and feet was noted. Physical examination was otherwise negative. The psychiatric diagnosis was psychosis with cerebral arteriosclerosis. Laboratory data showed negative serologic findings, a red blood cell count of 4,400,000; 81 per cent hemoglobin, and a white blood cell count from 8,600 to 10,400, with a normal differential. The urine revealed occasional casts and a 1 plus albumin, otherwise negative. The nonprotein nitrogen of the blood was 28 mg. per cent. The patient succumbed to bronchopneumonia 3½ months after admission.

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The final pathologic diagnoses were: Senility; senile plaques in brain; fibrosis of spleen, pancreas, and testes; brown atrophy of liver; amyloid in heart; bronchopneumonia; fibrinous pleuritis; acute passive congestion of liver; fibrous perisplenitis; accessory spleen.

The heart weighed 350 gm. Slight calcification was present at the bases of the aortic cusps, but the free margins were delicate. No gross scarring was observable. The coronary arteries, although tortuous, revealed only mild atheromatous deposits. The aorta was the seat of only slight atheroma. Arteriosclerosis was not considered as a significant part of the disease picture. The microscopic examination of the heart is discussed later. No amyloid was noted in any other organ. The other diagnoses need no comment.

Case 2

An 83-year-old man was admitted in a somewhat disoriented condition with a history of urinary difficulty, consisting of nocturia, difficulty in starting the stream, and dribbling, of uncertain duration. On physical examination the significant findings were some increased resonance in both lungs, moderate kyphosis, and scattered râles in the right lung. The heart was moderately enlarged to percussion, and a soft systolic murmur was present at the apex. The prostate was enlarged, symmetric, firm, nontender, and smooth. The blood pressure was 140/ 80 mm. Hg. Slight pitting edema of the lower extremities was present. Urine showed 3 plus albumin, no sugar, and many pus cells. The red blood count was 3,620,000, with but 38 per cent hemoglobin; the white blood cell count was 9,720, with a normal differential. Nonprotein nitrogen of the blood was 47 mg., creatinine 1.6 mg. per cent. The heart action was irregular. The electrocardiogram showed auricular fibrillation and diffuse myocardial damage. There was roentgenologic evidence of Paget's disease, involving the skull, spine, femora, and pelvis. The alkaline phosphatase value was 9.8 Bodansky units and the acid phosphatase, 1.6 units. The phosphorus was 3.8, and the calcium 7.5 mg. per cent. The patient was given numerous transfusions. Following these the red blood cell count was 4,000,000; the color index, 0.8. At operation, an extensive carcinoma of the bladder, of papillary type, was excised, and the prostate removed. Pathologic examination showed benign hyperplasia of the prostate, and papillary carcinoma of the bladder, transitional cell type. The patient did not do well after operation. His nonprotein nitrogen gradually rose to 172 mg. per cent with creatinine of 5, and he expired 14 days after operation.

The pathologic diagnoses following autopsy were: Recent operation (prostatectomy and removal of carcinoma of bladder); necrotizing cystitis; chronic pyelonephritis; generalized arteriosclerosis; calcific aortic sclerosis (Mönckeberg type), mild; myocardial hypertrophy and fibrosis; amyloid in heart; chronic passive congestion of liver; congestion and edema of lungs; Paget's disease of bone.

The heart weighed 470 gm. and was dilated in all chambers. Considerable calcification was present in the sinuses of Valsalva, and in the bases of the aortic cusps and mitral ring. The free margins of the valves were delicate. Extensive calcification and arteriosclerosis were

present in the coronary vessels, as well as in the aorta and major arteries. Microscopic study of the heart is discussed later. The left kidney weighed 70, the right, 180 gm. Both were coarsely granular, with obscuration of the corticomedullary junction, with severe fibrosis, and interstitial infiltrations of lymphocytes and plasma cells. Some acute inflammatory changes were present in collecting tubules and pelvic mucosa, but these were relatively slight. The skeletal changes were typical of Paget's disease but relatively inactive. No amyloid was noted except in the heart.

Case 3

The clinical record on case 3 is inadequate. The patient, an irrational 87-year-old woman, died 30 hours after admission. On entry her abdomen was markedly distended, the legs were edematous. Temperature on admission was 97° F. The clinical impression was arteriosclerotic heart disease. The red blood cell count was 4,900,000; hemoglobin, 14.1 gm.; white blood cell count 23,250, with 91 per cent polymorphonuclear leukocytes. Serologic findings were negative. Nonprotein nitrogen of the blood was 57; urea nitrogen, 32 mg. per cent. Paracentesis was performed and approximately 500 cc. of turbid fluid were removed. The patient remained irrational. Her temperature never rose above 97° F. and was recorded at 96° F. shortly before death.

The final pathologic diagnoses were: Generalized arteriosclerosis; calcific aortic sclerosis (Mönckeberg type); acquired bicuspid aortic valve; cardiac hypertrophy and dilatation; myocardial fibrosis; amyloid in heart; chronic passive congestion of lungs; amyloid in lungs (slight); pleural effusions; cardiac cirrhosis of liver; edema of ankles; infarct of spleen; generalized peritonitis, cause undetermined; slight bronchopneumonia; transitional cell carcinoma of vagina.

The two major disease sequences could not be brought into any definite relationship. The peritonitis was generalized and severe, but no cause therefor could be ascertained. The gastro-intestinal and genito-urinary tracts were anatomically intact. No primary focus of infection could be identified. The bronchopneumonic patches in the lungs did not appear antecedent or causal to the peritonitis. The splenic infarct was bland, and not septic. It was suggested that perhaps, in the patient's enfeebled condition, peritoneal infection might have occurred from the lumen of the bowel through an anatomically intact wall.

The circulatory system proved of great interest. The heart weighed 630 gm. The aortic valve was the seat of severe calcific sclerosis, most pronounced at the bases of the cusps. A bicuspid valve was produced. The free margins of the leaflets were pliable, although slightly thickened. Calcareous deposits at the bases of the mitral cusps also were present. Fine grayish streaking was visible in the myocardium. Microscopic findings are discussed later. The findings in liver and lungs were

indicative of long-continued cardiac insufficiency, with prominent chronic passive congestion, reaching the intensity of cardiac cirrhosis in the liver. In the lung small amounts of amyloid were noted in the thickened alveolar walls, but none was seen in any other organ. A small, recent, bland infarct of the spleen was considered to be arteriosclerotic in origin. The kidneys weighed about 120 gm. each and appeared excellently preserved.

An incidental finding was a small nodule, about 1.5 by 1 cm., situated in the posterior vaginal wall at the posterior fornix, which on section proved to be a papillary carcinoma of transitional cell type. It was superficial and did not invade the vaginal wall.

Case 4

The patient was a male, 93 years old, who had had two hospital admissions. On the first his complaints were dyspnea on exertion for about 3 weeks, progressive weakness for 3 months, and black stools for 2 weeks. The significant physical findings were: Enlargement of the heart to the left, with extrasystoles and a harsh apical systolic murmur. The blood pressure was 150/80 mm. Hg; red blood cell count, 2,550,000, with 43 per cent hemoglobin; white blood cell count, normal. Nonprotein nitrogen of the blood was 47 mg. per cent, but creatinine and sugar were normal. The electrocardiogram was interpreted as showing severe myocardial damage. Stool examinations showed occult blood. After repeated blood transfusions a gastro-intestinal x-ray series was made, showing an apparent gastric ulcer on the lesser curvature. The patient's condition improved after appropriate therapy, and he was discharged 17 days later. The clinical impression was: Bleeding peptic ulcer of stomach; organic heart disease with mitral insufficiency and mild congestive failure.

The patient was re-admitted 7 weeks later because of pain, redness, and swelling of the left leg, of 3 weeks' duration. He had been relatively well on digitalis in the interim. Examination showed thrombophlebitis of the left leg; slight auricular fibrillation; blood pressure, 150/76 mm. Hg; red blood cell count, 4,700,000, with 70 per cent hemoglobin; white blood cell count, 16,500, with 85 per cent polymorphonuclear cells. Nonprotein nitrogen of the blood was 24 mg. per cent. The patient was treated with dicumarol, the prothrombin time being regulated at 35 to 40 per cent of normal. The thrombophlebitis showed marked improvement. However, 13 days after the final admission, the patient died rather suddenly.

The pathologic diagnoses after autopsy were: Generalized arteriosclerosis; myocardial fibrosis; myocardial hypertrophy; amyloid in heart; dilatation of heart; chronic passive congestion of lungs; amyloid in lungs, slight; thrombophlebitis, left femoral vein; infarct of spleen; petechial hemorrhages of intestinal serosa and pelvis of kidney. No residuum of a peptic ulcer was encountered. No pulmonary embolus was present.

The heart weighed 420 gm. Considerable epicardial fat was noted. There was moderate dilatation of the chambers and some hypertrophy of the musculature. The valves were delicate, except for slight nodular thickening of the free edges of the mitral cusps, but the chordae ten-

dineae were delicate and inserted normally. The coronary arteries, patent throughout, were tortuous, but showed no calcification and only slight atheroma. Notation was made that they appeared extraordinarily well preserved for a man of this age. Dense fibrosis was noted at the tips of the papillary muscles, and a few minute, translucent, grayish dots and fine streaks were observed in the myocardium, especially of the septum. The aorta showed mild sclerotic changes. The microscopic findings in the heart are described later. The left femoral vein was occluded by a thrombus, and chronic inflammatory infiltrations were present in the thickened wall. The splenic infarct was small, measuring only 1.5 cm. across, and was recent. Moderate chronic passive congestion involved the lungs. Small traces of amyloid were seen in the thickened alveolar walls, but not in any other organ. The petechial hemorrhages of the intestinal serosa and renal pelvis were apparently agonal.

Case 5

The patient was a woman, 88 years old, who was admitted following a fall at home, in which she fractured her right hip. The fracture was inter-trochanteric, and comminuted. Physical examination otherwise was not remarkable, except for an apical systolic murmur. With the extremity in traction, the patient was placed on sulfonamide therapy and her general progress was satisfactory. The terminal episode, 3 months after admission, was ushered in by a sudden hemorrhage from the rectum. In spite of supportive therapy, she died within a few hours.

The pathologic diagnoses were: Generalized arteriosclerosis; occlusion of inferior mesenteric artery; infarction of distal transverse colon, sigmoid, and rectum; nephrosclerosis, arteriosclerotic type; old healed infarct of lung; amyloid in heart and lung; fibrous perisplenitis; portal cirrhosis of liver, mild; bullous emphysema, moderate; atelectasis, right middle and lower lobes; lymphocytic infiltration of adrenals; parovarian cysts, bilateral; old fracture of the right hip.

The heart weighed 230 gm. Fine grayish streaks were noted in the myocardium. The aortic valve showed slight calcareous deposits at the base of the cusps. The coronary vessels were severely sclerotic, but the lumina were only moderately narrowed. The microscopic findings are considered below. The aorta revealed extensive ulceration and calcification, and the ostium of the inferior mesenteric artery was occluded. The other major arteries were affected by severe atherosclerosis and calcification. The infarction of the distal colon, sigmoid, and rectum was characteristic. The lungs, with senile emphysema and some bullous formation, weighed only 190 and 150 gm. respectively. Some atelectasis also was present. One minute area of fibrosis, with fibrotic occlusion of a nearby artery, was interpreted as an old healed infarct. Traces of amyloid were present within the media of the

fibrotic blood vessels in this area. None was seen elsewhere, neither in other parts of the lung nor in other organs. The liver weighed 1000 gm. The surface was slightly granular, and microscopic examination showed a distortion of architecture by a mild excess of fibrous tissue, radiating irregularly from the portal spaces. The nephrosclerosis, with kidneys weighing 105 and 115 gm., was characteristically arteriosclerotic in type. In the pancreas small areas of fibrosis affected the interstitial connective tissue, but the islands were intact.

MICROSCOPIC STUDY OF HEARTS

These 5 cases appear to form a single group and may be considered together. The ages of the patients ranged from 83 to 93 years. The causes of death varied greatly. The weights of the hearts ranged from 230 to 630 gm. The heart weighing 630 gm. showed severe Mönckeberg's sclerosis of the aortic valve, with an acquired bicuspid valve, and the hypertrophy seemed a direct result of this. Three of the hearts with weights of 420, 470, and 630 gm. showed considerable interstitial fibrosis but the other 2 (230 and 350 gm.) revealed no significant fibrosis within the myocardium. Arteriosclerotic changes, generalized, were prominent in all but case 1, but less so in case 4 than in cases 2, 3, and 5.

The microscopic examination of the hearts revealed fundamental similarity. In all there were small patchy masses of hyaline material, situated in the interstitial tissue, and surrounding muscle fibers singly and in groups. This hyaline material in all specimens reacted positively with Congo red and with methyl violet. The latter stain gave intense metachromatic reactions, while the Congo red reaction was less strong. The hyaline substance also stained positively with ammoniacal silver, according to the method given below. The general pattern of the amyloid, in all cases, is illustrated in Figures 1 and 2. The hyaline material (Fig. 1) at first glance suggests fibrosis. In foci where the involvement is intense, the muscle fibers have disappeared or are markedly shrunken and atrophic, as if choked by the surrounding collars of amyloid. Careful examination, however, shows absence of fibrillar texture characteristic of collagen, while the deposits are hyaline. Differential staining reactions are, of course, conclusive.

Where variable amounts of interstitial fibrosis were present, amyloid was observed not only in immediate contact with muscle fibers but also as occasional small plaques and masses of hyaline material within the fibrosed zones. These deposits of amyloid within areas of fibrosis were readily recognizable in preparations stained with hematoxylin and eosin, and were differentially shown by specific stains. Methyl violet

was most useful in this regard. The heart of case 3 revealed the most marked interstitial fibrosis in this series.

It must be emphasized, however, that in none of these cases was the total amount of amyloid very great, and certainly not comparable to the massive deposits reported occasionally in the literature. The hypertrophy in the largest heart in this series, 630 gm., was explained by the aortic valvular disease. In all 5 cases the amyloid appeared to be an incidental finding. There was not sufficient evidence to attribute death to the deposition of amyloid.

In cases 3, 4, and 5 small amounts of amyloid were noted in the lungs. In cases 3 and 4, chronic passive congestion of the lungs was present, and the small amyloid masses were situated in the thickened alveolar septa. In case 5 the hyaline substance was seen within arterial walls in a zone of old infarction. In none of the cases was amyloid observed in any other organ.

AMYLOIDOSIS WITH PYELONEPHRITIS

A sixth case of atypical amyloid disease appears to be in a totally different category, but may be reported at this time.

Case 6

The patient, 70 years of age, speaking practically no English, was admitted in a critically ill condition, with nausea, vomiting, and severe abdominal pain of about 1 week's duration. The clinical impression was acute intestinal obstruction. Laboratory data showed a white blood cell count of 22,700, with 95 per cent polymorphonuclear leukocytes, and 4 plus albumin in the urine, with many pus cells. He had had previous surgical treatment, as evidenced by old abdominal scars. Although his physical condition was poor, immediate laparotomy seemed indicated. Severe adhesions were encountered and freed, releasing kinked and adherent loops of small bowel. An enormously distended gallbladder was noted. This was drained, and the abdomen closed. In spite of supportive postoperative therapy, the patient died 20 hours after admission.

It was found, subsequently, that the patient had had a number of hospital admissions in various hospitals, over a period of at least 12 years. He had had a resection of the cecum and ascending colon, with ileocolostomy, for carcinoma of the colon, verified pathologically. Apparently, he had a partial resection of the stomach, with anterior gastrojejunostomy at another hospital, but this record has not been verified. In the 12-year period there were numerous hospital admissions for vomiting, pain in the upper abdomen, dizziness, abdominal discomfort, anorexia, fatigue, chest pain, and vague symptoms. There was some evidence that over the past 12 years numerous urine examinations showed albumin and white blood cells, but no particular significance had been attached to them.

The pathologic diagnoses following autopsy were: Nephrolithiasis, right; severe subacute and chronic pyelonephritis; chronic ureterocystitis; atypical amyloidosis, involving kidneys, and blood vessels of numerous organs; arteriosclerosis of coronary arteries; healed infarct of heart; chronic passive congestion of lungs; atelectasis; emphysema;

scars of old operations (partial colectomy with ileocolostomy for carcinoma of colon, gastrojejunostomy with partial gastric resection); recent cholecystostomy and separation of adhesions.

The right kidney, intact, weighed 250 gm. It contained a large staghorn calculus, tightly surrounded by a rim of renal tissue. The dilated calyces contained abundant, greenish, purulent material. The persisting renal tissue, of greenish red color, showed severe reduction of the usual markings and of definition between cortex and medulla, and presented a somewhat mottled appearance. The right ureter had a diameter of 1.3 cm., with a dense, firm wall and a minute, slit-like lumen. The left kidney, weighing 220 gm., was granular, with a soft, bulging cut surface showing dulling but not obscuration of the renal markings. No hydronephrosis or calculi were present on the left and the left ureter appeared normal. The bladder was thin-walled, but with severe congestion of the mucosa. The prostate revealed slight hyperplasia. The heart weighed 370 gm., and showed an old healed infarct of the posterior left ventricular wall, extending into the septum. The coronary arteries exhibited extensive sclerosis and narrowing, but total occlusion was not discovered. Microscopic examination showed severe scarring and cellular infiltrations of the right kidney, with only slight fibrosis and pyelonephritis of the left. The right ureter and bladder revealed prominent chronic inflammatory changes and fibrosis. In both kidneys the glomeruli were involved by massive deposits of amyloid, of the usual subendothelial type (Fig. 3). In addition, the capsules of Bowman frequently were thickened by fibrous tissue, and many glomeruli were sclerosed and fibrotic without amyloid infiltrations. No amyloid was seen in relation to the tubules or interstitial connective tissue, but many of the small and medium-sized blood vessels contained lumpy hyaline masses in their walls. These masses stained positively for amyloid (Fig. 4). In the other organs amyloid was found in the blood vessel walls (similar to Figs. 4 and 5), in the heart, liver, gallbladder, gastro-intestinal tract, adrenal capsule (Fig. 5), ureter, and bladder. None was observed in the parenchymatous tissues of these organs, but only in the walls of arteries and veins. None was seen in relation to capillaries or sinusoids. No amyloid could be identified in any part of the lung, spleen, or pancreas. In the heart the amyloid was observed only within the walls of the coronary vessels. None was observed surrounding the muscle fibers, as in cases 1 to 5.

In summary, this case, with very long-standing pyelonephritis and nephrolithiasis, showed amyloidosis limited to the renal glomeruli and to arteries and veins in many different organs.

Classification of Amyloid Disease

Without attempting an exhaustive review of the literature, certain facts stand out clearly. The most common manifestation of amyloid disease occurs in the course of tuberculosis. Perla and Gross,[3] in a study of 1500 autopsies, reported 100 cases representing about 25 per cent of all patients dying of tuberculosis. In this form of the disease, the amyloid is most commonly distributed in the kidneys, liver, spleen, and adrenals; less frequently in one, two, or three of the above organs, but not in all.[4] Huebschmann [5] reported that in 8 of 9 consecutive autopsies on tuberculous patients with amyloidosis, the amyloid was found in the heart also. In addition, the amyloid may be found in traces in other organs, such as parts of the gastro-intestinal tract, pancreas, or salivary glands.[6] Thus there is a certain pattern of distribution, which I wish to call *typical,* that is, predominantly in the parenchyma of kidneys, spleen, liver, and adrenals, less frequently and with less intensity in certain other sites. This typical form of distribution, called secondary in the literature, is seen in the course of tuberculosis, osteomyelitis, pyelonephritis, lung abscess, carcinoma of stomach,[3, 7] carcinoma of lungs, leukemia,[3] tabes,[3] Hodgkin's disease,[7, 8] multiple myeloma,[9] rheumatoid arthritis,[3, 4, 10] thermal burns,[11] and others.

The use of the term secondary for this type is entirely misleading. It implies that, somehow, the associated disease (tuberculosis, for example) is the cause of the amyloid. A philosophic discussion of causality is scarcely relevant to the present paper. One must distinguish, however, a proximal or immediate cause, which is both necessary and sufficient (for example, prolonged local anoxia as a cause of infarction); and a more remote link in the causal chain (for example, generalized arteriosclerosis as a "cause" of infarction) which is neither necessary nor sufficient to produce the given phenomenon, but which may initiate or induce the proximal cause.

In this sense it is obvious that the proximal cause of amyloidosis is not known, in spite of considerable experimental study and chemical analysis. However, the statistical correlation between tuberculosis and secondary amyloidosis is too high to be ignored. One must assume, therefore, that tuberculosis, while not the direct cause, is probably part of the causal chain, provided that certain other unknown factors also are present.

The rôle of chronic suppurative disease in the production of amyloidosis is probably similar to that of tuberculosis. However, the position of conditions such as arthritis or Hodgkin's disease is more

questionable. There simply is not sufficient evidence to implicate these diseases as part of the causal chain, although they cannot arbitrarily be excluded. Judgment must be suspended.

This necessity for suspending judgment is emphasized by the rare case of amyloidosis, with essentially typical distribution, in which no other disease is observed. In various published cases,[12-16] for example, no associated disease was present, but the distribution of amyloid was approximately comparable to that seen in the so-called secondary type. Since we are ignorant of the true cause of amyloidosis, to call one group secondary and the other primary seems illogical. One might suggest that the true proximal (but unknown) cause was the same in both groups. In the alleged secondary group an associated disease might or might not be a remote factor of the causal chain; in the so-called primary group the causal chain is unknown throughout. In some instances, *e.g.*, case 2 of Dillon and Evans,[15] an associated disease (bacterial endocarditis) was present, but the authors nevertheless considered the case primary. In our state of ignorance such nomenclature is utterly confusing.

There is a second major category of amyloid disease which differs from the preceding in the sites of deposit of the amyloid. This category includes most of the so-called primary or atypical cases. Lubarsch,[6] and others, have discussed this group. The important criteria are: (1) Deposition of amyloid in unusual sites (heart, lungs, striated and smooth muscle, skin); (2) sparing of the usual or typical sites (spleen, kidneys); (3) absence of demonstrable cause. Inconstancy of staining reactions may or may not be present. The literature of these cases has been most admirably reviewed by Lindsay,[2] and repetition is unnecessary.

Since the cause of amyloid deposition is not known, classification by etiology is not possible; and since there is no constancy in the clinical picture, clinical considerations must be kept in the background. The simplest classification, taking into account the known factors, and not stressing hypothetic factors, would be on the basis of anatomic distribution. The following is suggested.

1. *Typical amyloidosis:* Deposition of amyloid in the usual sites (kidneys, spleen, liver, adrenal, etc.)
   a. Associated with other disease (as, tuberculosis, multiple myeloma, carcinoma, osteomyelitis)
   b. Not associated with other disease (rare, but occasionally reported as "primary")
2. *Atypical amyloidosis:* Amyloid, not following the usual or typical

distribution, found in one or many foci or organs, with or without symptoms

a. Associated with other disease or conditions (as, multiple myeloma, Hodgkin's disease, carcinoma, pyelonephritis, bronchiectasis, and the like)
b. Not associated with other disease (including most of the cases reported in the literature as primary amyloidosis, whether systemic or local)

For purposes of classification it is necessary to be somewhat arbitrary, and it is proposed that the parenchymatous involvement of liver, spleen, kidneys, and adrenals be considered *typical.* The addition of other organs (for example, the unusual cases of Gerber [17] and Edens [18]) should not be sufficient to remove it from this category. On the other hand, *atypical* should be applied to those cases in which liver, spleen, kidneys, and adrenals are spared, or in which only one of them is involved with extensive amyloid in less usual sites as defined by Lubarsch.[6] This is admittedly an arbitrary division, but the diversity of cases is so great that otherwise no simple schema is feasible.

The most widely accepted classification hitherto is that of Reimann, Koucky, and Eklund,[19] who defined four groups: Primary, secondary, amyloid with multiple myeloma, and tumor-forming amyloid. More logical is the classification of Rosenblum and Kirshbaum,[20] who divided amyloidosis into primary or idiopathic and secondary or symptomatic, with subdivisions in each group of diffuse or typical, and localized or atypical.

It is the contention of this paper that there is no warrant for the use of the terms primary and secondary. Lindsay [2] has already expressed objection to these terms, suggesting "that when the basic mechanism is known, primary amyloidosis will be classified as a 'secondary' type."

Amyloidosis, however, takes so many different forms that a few words of comment are in order. One of the most interesting groups is that associated with multiple myeloma. Of approximately 650 cases reported in the literature,[9, 21, 22] concomitant amyloidosis was noted in 41. Most often there is an atypical distribution, but sometimes, as far as can be determined from available data, the distribution is comparable to that seen after tuberculosis, designated typical in this paper. Reimann et al.[19] would call this group neither primary nor secondary, but would relegate it to a separate category. Similarly, so-called tumor-forming amyloidosis which may involve bones,[23, 24] upper respiratory tract,[25] or conjunctiva [26] need not have a separate niche apart from

other forms, but can be grouped with atypical amyloidosis. Some of these cases are associated with multiple myeloma,[21, 22] others show no associated disease. The classification proposed herein readily accommodates these cases. The skin disease lichen amyloidosis,[27-30] frequently reported in the dermatologic literature, is another example of atypical amyloidosis. There seems no need for separate categories or divisions for each of the many examples of localization of amyloid.

ATYPICAL AMYLOIDOSIS, ASSOCIATED WITH SENILITY

The first 5 cases reported in this paper appear to form a single group, all showing amyloid in the heart, with little or no localization elsewhere. The amount of amyloid was relatively small compared to that in some of the cases described in the literature,[2] and was found incidentally at autopsy. All of the patients were 80 years of age or over. They all showed other lesions adequate to explain death, and the only common feature was advanced age. These cases apparently are identical with the 3 reported by Ranström,[31] in patients 80, 81, and 88 years of age. The older literature contains a brief note by Beneke [32] reporting 6 similar cases in old individuals, but the ages are not given. The case of Beneke and Bönning [33] is probably in the same category, but again age and clinical data are not presented.

The conclusion seems justified, on the basis of my own and other cases, that the amyloid deposition in the heart without clinical symptoms, can be correlated with old age. It appears to be entirely misleading to consider such cases as primary. It is of interest that of the 5 cases, 4 were found in a period of 16 months, in a total of 193 autopsies. In this small series 19 patients were 80 years of age or over, giving a percentage of 21 for that age group. This suggests that the condition is far more prevalent than has hitherto been suspected.

There is independent evidence that under certain circumstances amyloid deposition is a function of age. In an investigation of amyloid in the genito-urinary tract, Bursell [34] studied deposition in the seminal vesicles and found an incidence increasing with age. Thus, in the age group 76 to 90, of 38 cases, amyloid was found in 13. Its deposition was not related to inflammation in the prostate. It is plausible that his cases belong in the same category of atypical amyloidosis associated with old age. In my own material, unfortunately, the seminal vesicles were not examined microscopically, but it is only a question of time until the correlation between the two groups is proved or disproved.

Why the advanced age group should show a significant amount of amyloid is not clear. It is possible, as suggested by Warren,[35] that atypical

amyloidosis represents a "widespread perversion of function" of the connective tissue, with dysfunction of fibroblasts. Possibly local ageing of fibroblasts may be a relevant factor. In this connection it is of interest to note the occasional finding of amyloid in sclerosed pancreatic islands of many diabetic subjects (atypical amyloidosis associated with diabetes), an observation indicating the importance of local factors.

ATYPICAL AMYLOIDOSIS ASSOCIATED WITH PYELONEPHRITIS

In the sixth case reported here, with a massive nephrolithiasis and severe pyelonephritis, the amyloid showed a curious localization. Its parenchymatous distribution was limited to the renal glomeruli, but in addition it was found in the walls of small blood vessels in many organs, including the heart. This distribution is unusual, and deserves the designation of atypical. We may assume that the concomitant pyelonephritis was the most significant associated disease. It is reasonable to designate this case as atypical amyloidosis associated with pyelonephritis. It is plausible that the infection, in this instance, had the same causal importance that tuberculosis or osteomyelitis has in typical amyloidosis. The peculiar localization is not explained. (The case suggests some similarities to that of Binford.[36])

Three important unsolved problems haunt the subject of amyloidosis: (1) Why does amyloid disease usually take a typical distribution, but occasionally an atypical localization? (2) What is the true proximal cause (or causes) of amyloid production (as contrasted with inciting or mediating factors or associations, such as the familiar tuberculosis)? (3) What, if any, are the essential chemical differences between the typical and atypical amyloid? It is probable that a thorough-going answer to any of these problems will automatically solve all of them.

Staining of Amyloid with Silver

Variations in the staining properties of amyloid have long been noted. The atypical form, especially, shows much inconstancy in respect to the usual tinctorial reactions. Hass and Schulz [37] have demonstrated the chemical complexity of the usual or atypical amyloid. Similar work has not been done with the atypical forms. Adequate chemical analysis may well display many differences. Meanwhile the tinctorial, or crudely histochemical studies must be relied on.

That amyloid can be impregnated with silver first came to my attention while studying with the late Dr. Pio del Río-Hortega. In the course of impregnating reticulin fibers of the spleen, some amyloid which was pres-

ent stained beautifully, in a tone different from either collagen or reticulin. This impregnation aroused no interest or special comment, and was accepted in his laboratory as a well known phenomenon. In the course of subsequent studies on silver impregnations of the nervous system, a simple method was adapted for the differential exhibition of senile plaques and Alzheimer strands.[38,39] This method, based on one of Hortega's nuclear stains, was found to impregnate amyloid with facility and clarity. A brief resumé of the method is given.

Thin frozen sections are washed in water, and placed in an ammoniacal silver solution. To 5 cc. of 10 per cent silver nitrate, there is added, dropwise, enough ammonia to produce and then just dissolve the characteristic precipitate. Then 6 to 8 cc. of sodium carbonate solution are added. Of crystalline sodium carbonate, I used a 5 per cent strength; of the anhydrous form, 3.5 per cent. Or, saturated lithium carbonate may be used as the added alkali. The resulting solution is diluted to 75 cc. with distilled water. To about 10 cc. of this solution, a few drops of pyridine are added and the solution gently heated in a small, covered beaker and lightly agitated until the sections turn a tobacco-brown. The temperature should not exceed about 45° C., which can be tested by applying the bottom of the beaker to the back of the hand. The brown sections are then washed in sodium thiosulfate ("hypo"), followed by water, and are mounted without toning. *No formaldehyde or other reducing agent is employed at any point.* The method gives indifferent results in paraffin sections, which can be kept in the silver solution in the paraffin oven until they turn a rich brown. This varies from ½ to 2 hours. The staining is far more diffuse than with frozen sections, and muscle cytoplasm is too deeply impregnated. Paraffin sections are not recommended.

Examples of frozen section impregnation with this method are shown in Figures 2, 3, 4, and 6. The amyloid stains deeply, but nuclei and lipochrome pigment also are deeply impregnated. Cytoplasm is frequently stained a light brown, and a few wisps of collagen may impregnate. Sometimes protein casts in the lumina of renal tubules stain with moderate intensity. Good silver impregnations were obtained in all 6 cases reported here. The results are permanent. Preparations from case 1 are vivid after a lapse of 6 years. In Figure 6 is shown an amyloid liver from a case of tuberculosis, to illustrate the staining of so-called secondary amyloid.

The method is more cumbersome than the methyl violet or Congo red reaction, and is not suggested as a substitute. It is presented as showing an interesting chemical property of amyloid, namely, an intense argyrophilia.

The reaction of amyloid with ammoniacal silver offers a new tool. The method is reported, not as a substitute for the simpler staining technics, but as a hitherto undescribed property of amyloid. It was previously shown in studies on senile brains [39] that the intracellular, Alzheimer strands as well as the interstitial senile plaques were strongly argyrophilic. That is, ammoniacal silver, facilitated by gentle heat, would impregnate these structures with regularity and intensity without

the use of any reducing agent. Nuclear material and lipochrome pigments also were regularly impregnated by the same method. Nerve fibrils and some connective tissue fibrils might be inconstantly and lightly stained. In the studies on amyloid the staining is not entirely selective, for, as can be seen in the illustrations, nuclei and lipochrome stain deeply. In general there is very good differentiation between amyloid and collagen, but occasionally some strands of heavy collagen will darken markedly with this technic. Further work is needed to elucidate the relationships and to correlate argyrophilia with other tinctorial properties.

Summary

The present-day classification of amyloid disease, with its distinction of primary and secondary, involves the user in inevitable inconsistencies. A new classification is proposed, based on typical or atypical distribution, and on association or lack of association with other diseases or conditions. With this classification, a new category, "atypical amyloidosis associated with senility," is presented. Five such cases are described, in subjects over 80 years of age in whom the amyloid was present almost exclusively in the heart, and only as an incidental autopsy finding. A further case of a different category, atypical amyloidosis associated with pyelonephritis, also is presented.

A newly described chemical property of amyloid is its ability to combine with ammoniacal silver without the use of any reducing agent. A simple technic is described for the application of this method, which proves to be a useful tool for the discovery and identification of this substance.

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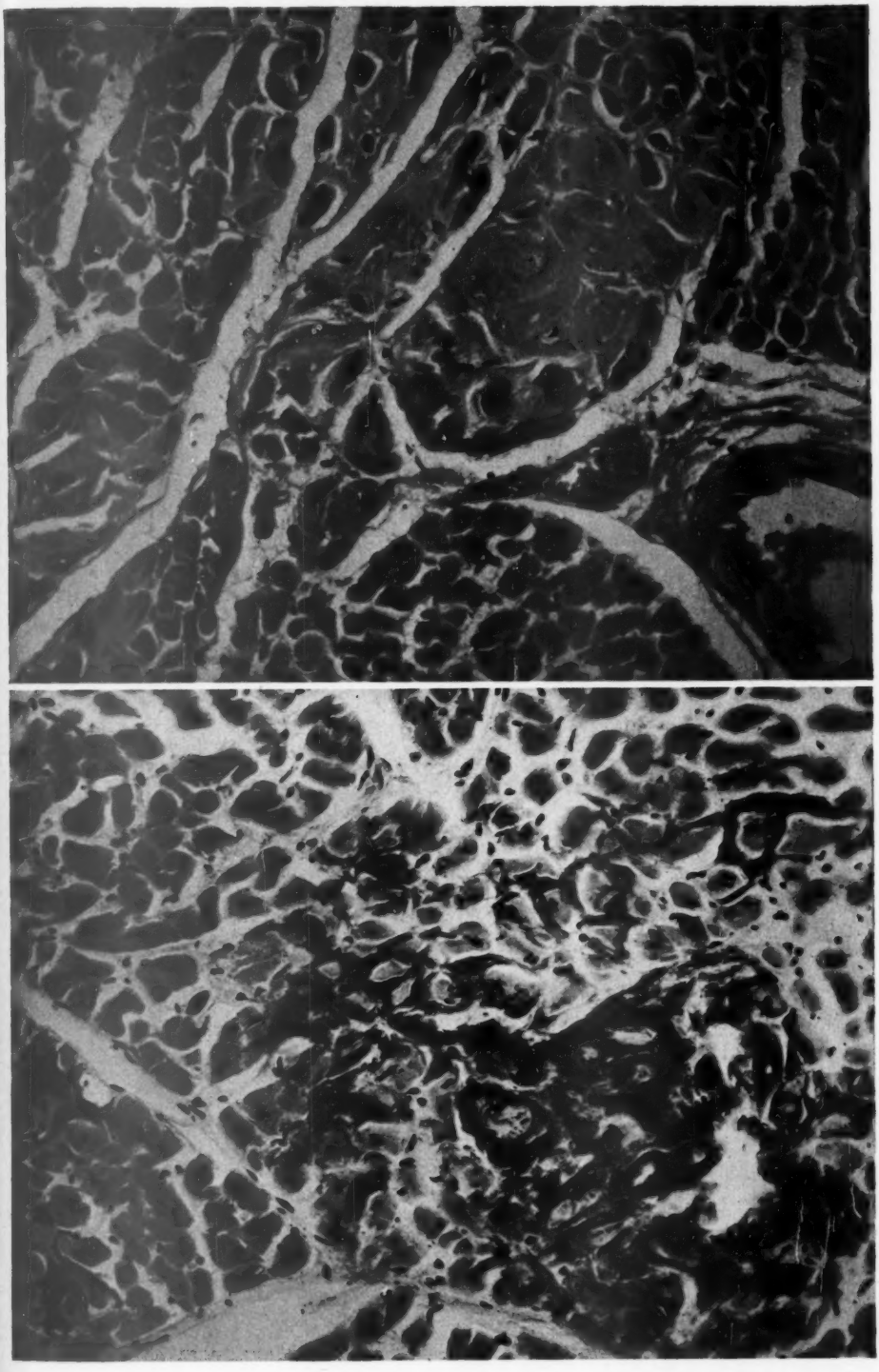
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 178

FIG. 1. Myocardium, case 4. Patches of amyloid occupy the interstitial tissue, replacing many muscle fibers and surrounding others in ring-like fashion. Many of the surrounded fibers are atrophic. Hematoxylin and eosin stain, paraffin section. × 600.

FIG. 2. Myocardium, case 4. The amyloid appears as dark-staining, lumpy masses surrounding some individual muscle fibers and replacing others. The interstitial connective tissue is essentially unstained, but nuclei and lipochrome pigment are impregnated. Silver impregnation, frozen section. × 600.



King Atypical Amyloid Disease

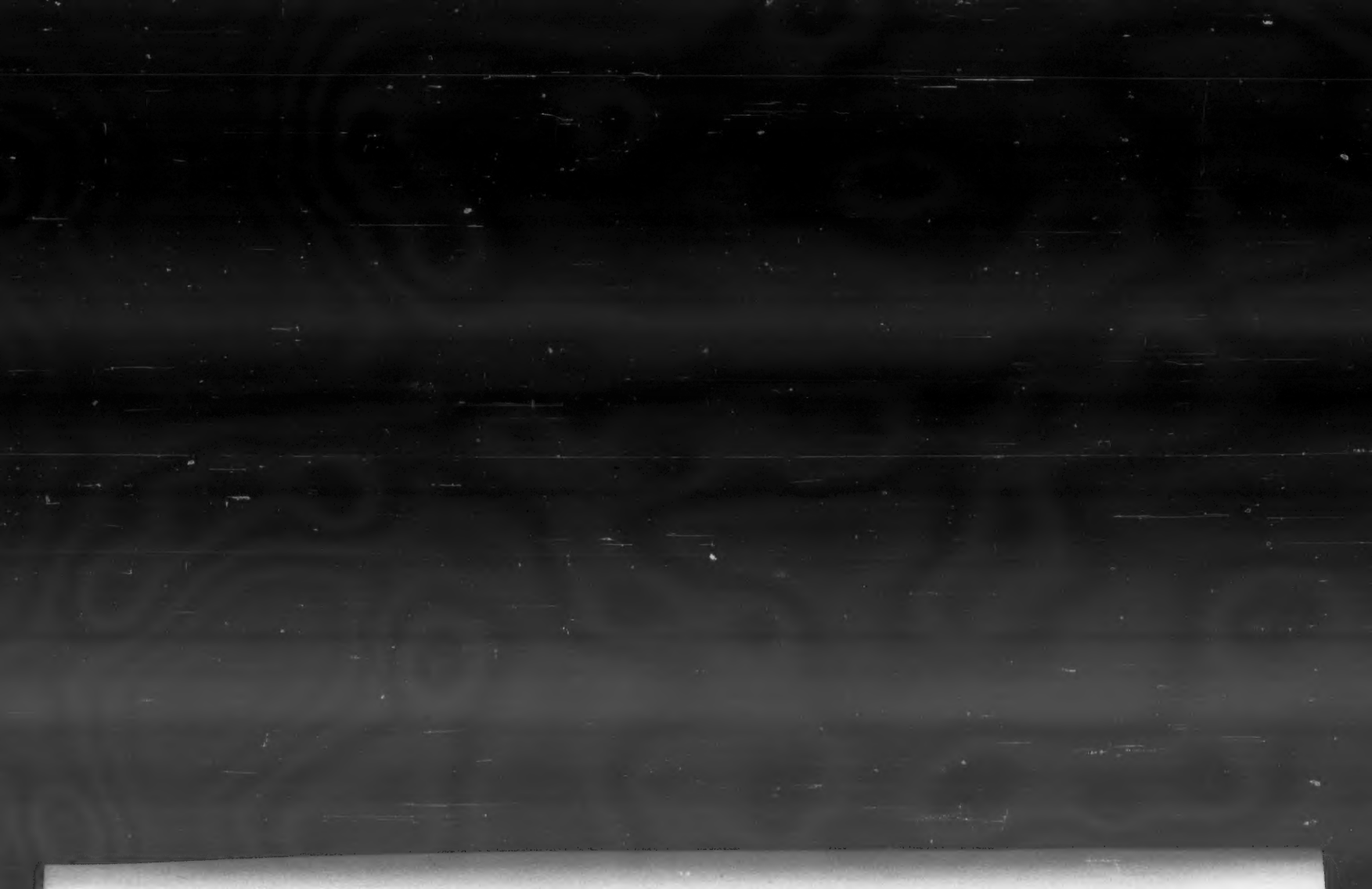
PLATE 179

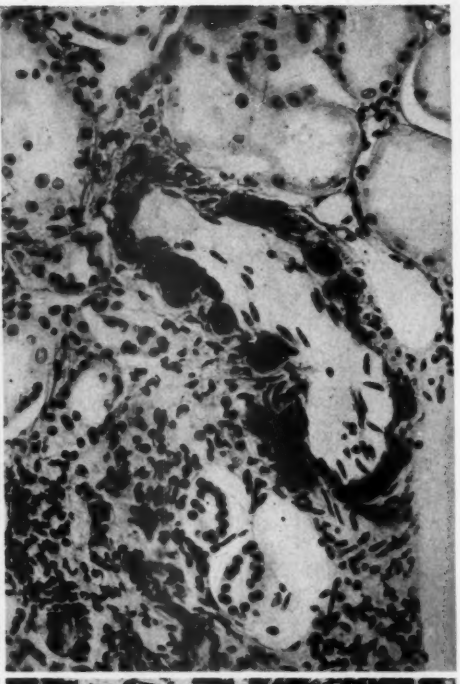
FIG. 3. Kidney, case 6. Amyloid within the glomeruli is heavily stained, and contrasts well with the connective tissue of the thickened Bowman's capsules. Nuclei are well shown. Silver impregnation, frozen section. × 500.

FIG. 4. Kidney, case 6. Amyloid is seen within the walls of a small blood vessel. Silver impregnation, frozen section. × 500.

FIG. 5. Adrenal, case 6. The amyloid in one of the periadrenal blood vessels is well shown. The general appearance after Congo red staining is similar to that seen with silver impregnation. No amyloid was present in the adrenal gland proper. Congo red and hematoxylin, paraffin section. × 500.

FIG. 6. Amyloid liver from a case of tuberculosis. In the material studied to date, there is no difference in reaction to silver between typical and atypical amyloidosis. At this magnification nuclei are visible as small dots. The extensive amyloid distribution is well shown. Silver impregnation, frozen section. × 55.





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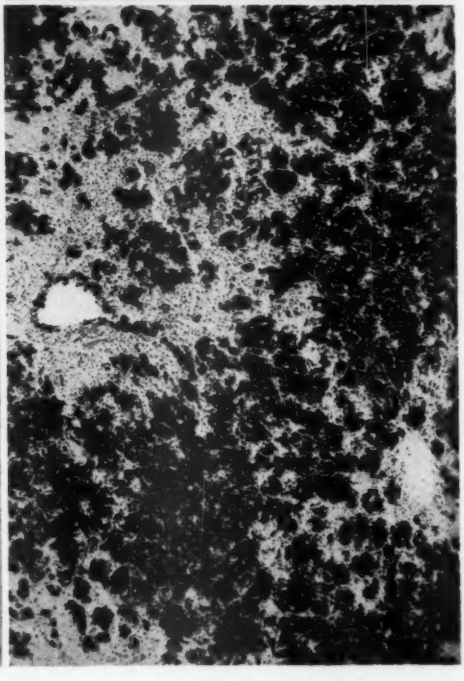

6

OBSERVATIONS IN GUINEA-PIGS FOLLOWING INJECTION OF SPECIFIC HEMATOPOIETIC SUBSTANCES DERIVED FROM URINES OF HUMAN LEUKEMIC SUBJECTS *

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In a previous report,[1] the effects of the injection of extracts of beef liver into guinea-pigs were described. The theoretic considerations that prompted the investigation and a brief summary of the literature were given. This report is concerned with a description and evaluation of the observations in guinea-pigs of the effects of extracts of urines of leukemic subjects.

MATERIALS AND METHODS

Thirty-three young male guinea-pigs were used. Weights ranged from 205 to 520 gm. Two animals, however, weighed 700 and 780 gm., respectively. These animals were standardized and observations made as described in the preceding paper.[1]

Urinary extracts were prepared in the following manner. Urines of patients with proved myeloid or lymphoid leukemia were collected in bottles containing chloroform and pooled according to type. The urine was adjusted with hydrochloric acid to pH 1 to 2, heated to boiling for 5 to 10 minutes, and then extracted with chloroform. The chloroform extract was concentrated, saponified in 2N sodium hydroxide solution, and extracted with ether to remove the neutral materials. The alkaline solution was saturated with carbon dioxide and extracted with ether to remove phenolic materials, then acidified with hydrochloric acid and re-extracted with ether. The ether extract was evaporated to dryness and extracted with petroleum ether. The petroleum ether extract was extracted with methanol to remove benzoic materials and made into lead salts in a hot alcoholic solution, which was then filtered. The alcohol-insoluble lead salts were extracted with ether to remove ether-soluble lead salts. The ether-insoluble lead salts were regenerated to acid form, dissolved in acetone, and kept at —20°C. for crystallization. They were then filtered to remove crystals of palmitic and/or stearic acids. The filtrate was concentrated and separated by succination into carbinols and noncarbinols. Extracts were then suspended in normal butyl succinate so that 1 cc. of extract suspension was equivalent to 1 l. of the original urine. One chloroform-extracted carbinol sample

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was suspended in cottonseed oil and 1 cc. of that suspension was equivalent to 1.33 l. of the original urine.

A second type of extraction, producing "ether extracts," was prepared in the following manner. Urines were pooled as described above, 2 per cent potassium hydroxide added, and the urine was then boiled and refluxed with steam under nitrogen for 4 hours. Upon cooling, neutral substances were removed with three chloroform extractions. The alkaline solution was acidified with sulfuric acid and three extractions with ether were made to remove the acidic substances. The extract was washed with water until neutral and then concentrated by steam heat under vacuum. The extract was suspended in normal butyl succinate so that 1 cc. of extract was equivalent to 1 l. of the original urine.

In order to insure potency of the extracts tested, the carbinol fractions used were those made from urines of patients with lymphoid leukemia. Similarly, the noncarbinol fractions used were those derived from the urines of patients with myeloid leukemia.

Description of Pathologic Changes

The gross and histopathologic changes observed were essentially the same as those noted in the preceding paper.[1]

Observations

In general, the local reactions to the urinary extracts were more severe than those observed with liver extracts. The animals appeared more ill. Ruffled fur, lethargy, and somewhat stertorous breathing were common. Ulceration of the hind feet occurred after the larger doses. The peripheral blood picture was not significantly altered except in occasional animals in which anemia was marked. In such, normoblasts and occasional myelocytes were noted in the smear. Weight loss during the course of the experiment was usual. In 21 animals spontaneous death terminated the experiment.

Carbinol (Lymphoid) Chloroform Fraction from Urines of Patients with Lymphoid Leukemia

Two different samples of chloroform extract were used. One sample was suspended in cottonseed oil. Normal butyl succinate was the medium used for the second fraction (Table I.)

The cottonseed suspension in doses of 0.2 cc. was injected, daily, intramuscularly into alternate hind limbs of 3 young male guinea-pigs weighing 239, 250, and 263 gm., respectively. Each animal received a total dose of 1.8 cc., representing 6.0 l. of the original urine. Periph-

eral blood findings remained normal. The curve of gain in weight was flattened slightly. After 0.8 cc. of extract, ulceration of the plantar surface of the right hind foot was noted in one animal. All animals showed some ruffling of hair, but appeared well otherwise. The experiment was terminated 7 and 9 days after the final injection. Two guinea-pigs were observed to have lymphoid responses, the other animal was negative.

Six male guinea-pigs weighing 240 to 300 gm. and one male weighing 700 gm. were given daily intramuscular or subcutaneous injections of

TABLE I

Results of Injection of the Carbinol (Lymphoid) Chloroform Fraction from Urines of Patients with Lymphoid Leukemia

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent urine	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*l.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
74	700	10.25	10.25	20	0	D	5.35	5.8	16.0	12.7	+ L
81	263	4.5	4.5	12	1	D	5.2	5.0	22.7	19.0	None
83	240	0.5	0.5	2	0	D		4.98		13.0	None
84	285	6.0	6.0	15	1	D	5.15	4.4	11.5	13.5	+++ L
86	255	5.25	5.25	13	0	D	5.3	4.8	12.3	15.0	++ L
87	300	1.25	1.25	6	1	D	5.1	5.3	12.6	12.6	+ L
88	280	5.5	5.50	13	1	D	5.2	5.4	14.6	30.0	+ L

Key: L = lymphoid reaction; D = died.

the butyl succinate suspended fraction. Initial dosage was 0.25 cc. This was increased every 5th day by 0.25 cc. until each animal was receiving 1.0 cc. daily. Injections were continued until death. Total dosage varied from 0.5 to 6.0 cc. except for the largest animal which received 10.25 cc. After 2 weeks of injections and a total dose of 5.25 cc., the left hind foot of one animal showed gangrenous changes which progressed until death 2 days later. The 8th day after the initial injection, all animals had ruffled fur and appeared to be ill. The hind limbs (sites of injection) became indurated. All animals lost weight during the experimental period. Peripheral blood changes were not significant. The animals survived from 2 to 20 days after the initial injection. Five died within 15 days, the 6th on the 20th day. Four animals had enlarged cervical nodes. The thymus was prominent in all. Two presented enlarged mesenteric nodes and in one the right inguinal node was enlarged. In the 4 animals with enlarged lymph nodes, lymphoid hyperplasia and infiltration of the cervical nodes (Fig. 1), and to a lesser extent of the liver (Fig. 2), adrenal, kidney, and lung (Fig. 3), were noted. The spleen was involved in only one animal (Fig. 4).

The bone marrow could not be studied adequately because of technical difficulties. The remaining 3 were negative except for some round-celled infiltration of the medulla of the adrenals and of the kidneys. The 4 positive animals received 5.25 cc. or more of extract.

Carbinol (Lymphoid) Ether Fraction from Urines of Patients with Lymphoid Leukemia

Three young male guinea-pigs weighing 275 to 430 gm. were given daily injections of 0.5 cc. of carbinol ether extract intramuscularly in alternate thighs. The total doses were 1.5, 2.0, and 4.5 cc. Death occurred 4, 5, and 13 days after the initial injections. The animal receiving the largest dose showed enlargement of the cervical lymph nodes and lymphocytic hyperplasia of the cervical, axillary, and mesenteric lymph nodes. The spleen was hyperplastic and infiltration of the bone marrow was observed (Fig. 6). Another animal showed lesser but similar changes in the spleen, adrenals, and lung, while the third revealed round-celled infiltration of the bone marrow but myeloid changes in the cervical nodes, spleen, adrenals, and lung.

Two animals were given a different sample of similarly prepared extract in the manner described above. A total dose of 4.0 cc., equivalent to 4.0 l. of urine, was injected. The animals appeared ill after the fourth injection and one developed bilateral gangrene of the hind limbs. They were sacrificed 8 and 20 days after the last injection. The bone marrow of both animals revealed round-celled infiltration, but the cervical nodes, spleen, liver, adrenals, and lung showed mild to moderate degrees of myeloid infiltration and metaplasia.

Noncarbinol (Myeloid) Chloroform Fraction from Urines of Patients with Myeloid Leukemia

Six male guinea-pigs ranging in weight from 382 to 780 gm. were injected subcutaneously with daily doses of 0.25 cc. of the noncarbinol chloroform fraction (Table II). Every 5th day the dose was increased by 0.25 cc. until 1.0 cc. was given daily. Subcutaneous injection caused indurated and partially ulcerated areas in the skin, and the intramuscular route was used when this occurred. After 9 days of injections, all 4 smaller animals had gangrenous and ulcerative changes of the toes of the hind feet. The animals were clinically ill. There was progressive deterioration, and loss of weight was noted in all. Severe anemia in one animal and mild anemia in 2 others was observed at this time. There was concomitant leukocytosis in all 3. In the guinea-pig showing the marked anemia, normoblasts and myelocytes were seen in the periph-

eral blood. There was one anaphylactoid death during the course of the experiment. Five animals died and one was sacrificed 20 to 35 days after the initial injection. The total dosage varied from 8.75 to 11.75 cc. of extract, representing 8.75 to 11.75 l. of urine. Peritoneal irritation was observed in 2 animals. The cervical nodes were not enlarged. The thymus was difficult to identify. In 5 animals the liver was enlarged but of normal color. Four spleens were strikingly enlarged and in these animals the adrenals were likewise enlarged. Testicular atrophy was noted in 5 instances. Marked necrosis and suppuration were present in all cases at the various sites of injection. Microscopically, myeloid metaplasia and marked infiltration of imma-

TABLE II

Results of Injection of the Noncarbinol (Myeloid) Chloroform Fraction from Urines of Patients with Myeloid Leukemia

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent urine	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*l.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
75	780	11.75	11.75	24	0	D	5.2	5.8	9.4	14.0	++++ M
76	700	11.75	11.75	35	11	S	5.45	6.5	5.6	25.0	++++ M
77	472	8.75	8.75	20	0	D	4.9	4.3	9.3	19.5	++++ M
78	465	11.75	11.75	25	1	D	5.5	2.97	8.3	29.6	+++ M
79	425	9.75	9.75	22	1	D	5.01	4.3	8.6	18.6	+++ M
80	382	11.75	11.75	26	2	D	5.6	4.3	9.0	12.6	++ M

Key: M = myeloid reaction; S = sacrificed; D = died.

ture myeloid elements in the lymph nodes, spleen, liver (Figs. 7 and 8), adrenals, and lung (Fig. 9) were observed in 5 of the 6 guinea-pigs.

Hyperplasia of myeloid elements of the bone marrow was present in all (Fig. 10).

Noncarbinol (Myeloid) Ether Fraction from Urines of Patients with Myeloid Leukemia

There were two samples of noncarbinol ether extract identically prepared, but injected at different times. Each cc. of extract represented 1 l. of urine. Two guinea-pigs were used for each assay. All 4 animals were males and varied in weight from 400 to 520 gm. Daily intramuscular injections of 0.5 cc. were given.

In the first assay, one animal received 3.5 cc. and the other, 4.0 cc. Both lost weight and were clinically ill. One became anemic, but no immature cells were noted in the peripheral blood of either guinea-pig. One died 10 days after the first injection, the other was sacrificed 12 days later. Peritoneal reaction to the extract was noted in both. The

cervical nodes of one animal were enlarged. Induration and necrosis of muscle at the sites of injection were moderate. Microscopic examination disclosed myeloid metaplasia and myeloid infiltration of the spleen, liver, adrenals, and kidneys, with hyperplasia of the bone marrow. The cervical nodes of one guinea-pig were similarly involved.

In the second assay, 2 guinea-pigs received a total dose of 4.0 cc. They remained clinically well until sacrificed 10 and 22 days after the final injection. The peripheral blood was unchanged. Gross examination revealed only mild induration at the sites of injection. Microscopic examination was negative except for a mild myeloid response in the bone marrow of both animals and in the spleen of one.

Normal Butyl Succinate Control

Three male guinea-pigs weighing 260 to 368 gm. were chosen as controls (Table III). They were given daily alternate subcutaneous

TABLE III
Normal Butyl Succinate Control

Guinea-pig no.	Initial weight	Extract, total dose	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
			Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	*cc.*	*days*	*days*		*millions*	*millions*	*thousands*	*thousands*	
89	320	12.00	33	11	S	5.2	5.10	10.5	9.25	+ L
90	368	12.00	33	11	S	5.35	5.05	8.8	10.5	+++ L
93	260	12.00	23	1	D	5.05	4.95	11.0	14.0	± L

Key: L = lymphoid reaction; S = sacrificed; D = died.

and intramuscular injections. Initially, 0.25 cc. was given and the dose was increased by a like amount every 5th day until the daily dose was 1.0 cc. All 3 animals received a total dose of 12.0 cc. The first 6.0 cc. was well tolerated without abnormal clinical or hematologic signs. With larger doses, ruffled fur and breakdown of the sites of injection were noted. In one animal, gangrene of the left foot was observed. Weight was maintained during the injection period and then weight gains were noted. One animal lost weight after 10.0 cc. was given and died 3 days later. The other animals were sacrificed 11 days after the final injection. No side reactions were observed during the course of the experiment. Gross pathologic changes were limited to mildly enlarged cervical nodes in all animals and moderate induration and necrosis of the tissue at the sites of injection. Microscopic examination revealed mild to moderate lymphoid stimulation of the lymph nodes and spleen. The lungs of 2 animals were moderately involved. The bone marrows of all 3 guinea-pigs were negative.

Discussion

We do not believe that we have produced leukemia, as we recognize the spontaneous form in animals and man. We have been able to produce leukemia-like lesions of either the lymphoid or myeloid type by the injection into guinea-pigs of substances of the carbinol or noncarbinol type, respectively. The fractions used are obviously crude. To what extent the menstruum is influencing the final result is difficult to state, but it is noteworthy that animals receiving carbinol (lymphoid) extract suspended in cottonseed oil manifest lymphoid stimulation. Animals receiving cottonseed oil alone tend to show myeloid stimulation if any response is elicited. Conversely, the noncarbinol (myeloid) fraction suspended in normal butyl succinate may show the most marked myeloid metaplasia and infiltrative response, while the succinate alone is lymphoid stimulating. In general, our results confirm those of Miller and his group [2] and the report of Heinle and his coworkers.[3]

Table IV

Frequency of Organ Involvement Following Use of the Carbinol (Lymphoid) Chloroform Extracts from Urines of Patients with Lymphoid Leukemia

	Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
Suspended in cottonseed oil	± L	1		3	2			3
	L	1	1			1	1	
	None		2		1	2	2	
Suspended in normal butyl succinate	± L	1		4	2	4	1	
	L	4	1	1			2	
	None	2	6	2	5	3	4	

Key: L = lymphoid reaction.

Using both beef liver and urinary extracts, it was found that the noncarbinol fractions produce the more striking lesions. The separation of extract into carbinol and noncarbinol fractions is possible, but in our hands three times the equivalent dose of urine (in liters) has been necessary to produce lesions comparable with those described by Miller and his group.[2] We have found the fractions obtained by crude chloroform extraction to be more potent than the ether-extracted or the water-soluble forms.

The frequency of organ involvement is shown in Tables IV, V, VI, and VII. The lesions of the carbinol (lymphoid) fractions were most frequent in the lymph nodes, liver, lung, kidney, and bone mar-

TABLE V

Frequency of Organ Involvement Following Use of the Carbinol (Lymphoid) Ether Extracts from Urines of Patients with Lymphoid Leukemia and Suspended in Normal Butyl Succinate. (The Results from Two Extracts, Prepared Identically, Are Shown in the Upper and Lower Portions of the Table.)

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± L				1	1		3
L	1	2				1	
± M	1				1		
M		1				1	
None	1		3	2	1	1	
L							2
± M	2		1	1	1		
M		2	1		1	1	
None				1		1	

Key: L = lymphoid reaction; M = myeloid.

row. The animals stimulated by the noncarbinol (myeloid) fractions showed lesions most frequently in the spleen, bone marrow, liver, lymph nodes, and adrenal cortex. The tables indicate pulmonary involvement in the myeloid reaction. In the majority of sections this took the form of a decrease or absence of normally present lymphoid tissue. Another point of interest not shown is the amount of thymic tissue present. We had no difficulty in identifying and removing the thymus of the carbinol (lymphoid) animals; it was often extremely difficult to do so in the noncarbinol (myeloid) group.

It was quite evident throughout the entire experiment that stimulation of myeloid tissue caused a reduction of lymphoid tissue in organs where lymphoid tissue was normally present and abundant, and vice

TABLE VI

Frequency of Organ Involvement Following Use of the Noncarbinol (Myeloid) Chloroform Extract from Urines of Patients with Myeloid Leukemia, Suspended in Normal Butyl Succinate

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± M				1	1	2	3
M	5	5	5	2	4	3	3
None	1	1	1	3	1	1	

Key: M = myeloid reaction.

versa. The peripheral blood, however, did not share in this relationship. In only one animal (guinea-pig 78, Table II) were immature myeloid cells, myelocytes, and nonsegmented neutrophils found in the peripheral smear. This was true in spite of evidence of myeloid stimulation in all bone marrows of guinea-pigs given noncarbinol (myeloid) fractions of urinary extract.

In the previous report [1] we commented on the cellular infiltrations observed at the sites of injections. It was again observed that the noncarbinol (myeloid) fraction of urinary extract produced marked granu-

TABLE VII

Frequency of Organ Involvement Following Use of the Noncarbinol (Myeloid) Ether Extracts from the Urines of Patients with Myeloid Leukemia and Suspended in Normal Butyl Succinate. (The Upper and Lower Portions of the Table Show the Results from Two Extracts Identically Prepared.)

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± M	1		1	2	1	1	1
M		2	1		1		1
None	1					1	
± M		1					2
M							
None	2	1	2	2	2	2	

Key: M = myeloid reaction.

locytic reaction with occasional areas of suppuration. In the carbinol (lymphoid) stimulated animals, few inflammatory cells were seen. The local inflammatory reaction was greater in the animals receiving urinary extract than in those receiving beef liver extract. At times this reaction was so marked as to be followed by necrosis and gangrenous changes. This was more frequent in animals receiving larger doses.

SUMMARY

We believe that our results indicate that the urines of patients with leukemia contain some substance or substances which are extractable and separable by the methods described. Depending upon the method used, these extracts are capable of producing in guinea-pigs a specific lymphoid hyperplasia and infiltration with the carbinol fraction, and myeloid hyperplasia and infiltration with the noncarbinol portion. In either case, the chloroform extraction method produced the most potent fractions. The lesions produced in the animals were clinically and pathologically dissimilar to spontaneous leukemia. Further confirma-

tion of the reciprocal relationship between lymphoid and myeloid tissues was observed.

The question of the relationship of substances such as native proteins, normal butyl succinate, and the carbinol extracts of beef liver to the fractions obtained from the urines of patients with lymphoid leukemia is yet to be defined. Similarly, the relationship of the myeloid stimulators, the nucleic acid derivatives, cottonseed oil, and the non-carbinol extracts of beef liver to the fractions obtained from the urines of patients with myeloid leukemia is of interest.

It is our belief that our data justify continuation of investigation. Efforts to purify and concentrate the active factors involved are being pursued.

Urinary extracts were prepared by Dr. Frank Stirn and Dr. E. C. Yen at the Lederle Laboratories, Pearl River, N.Y.

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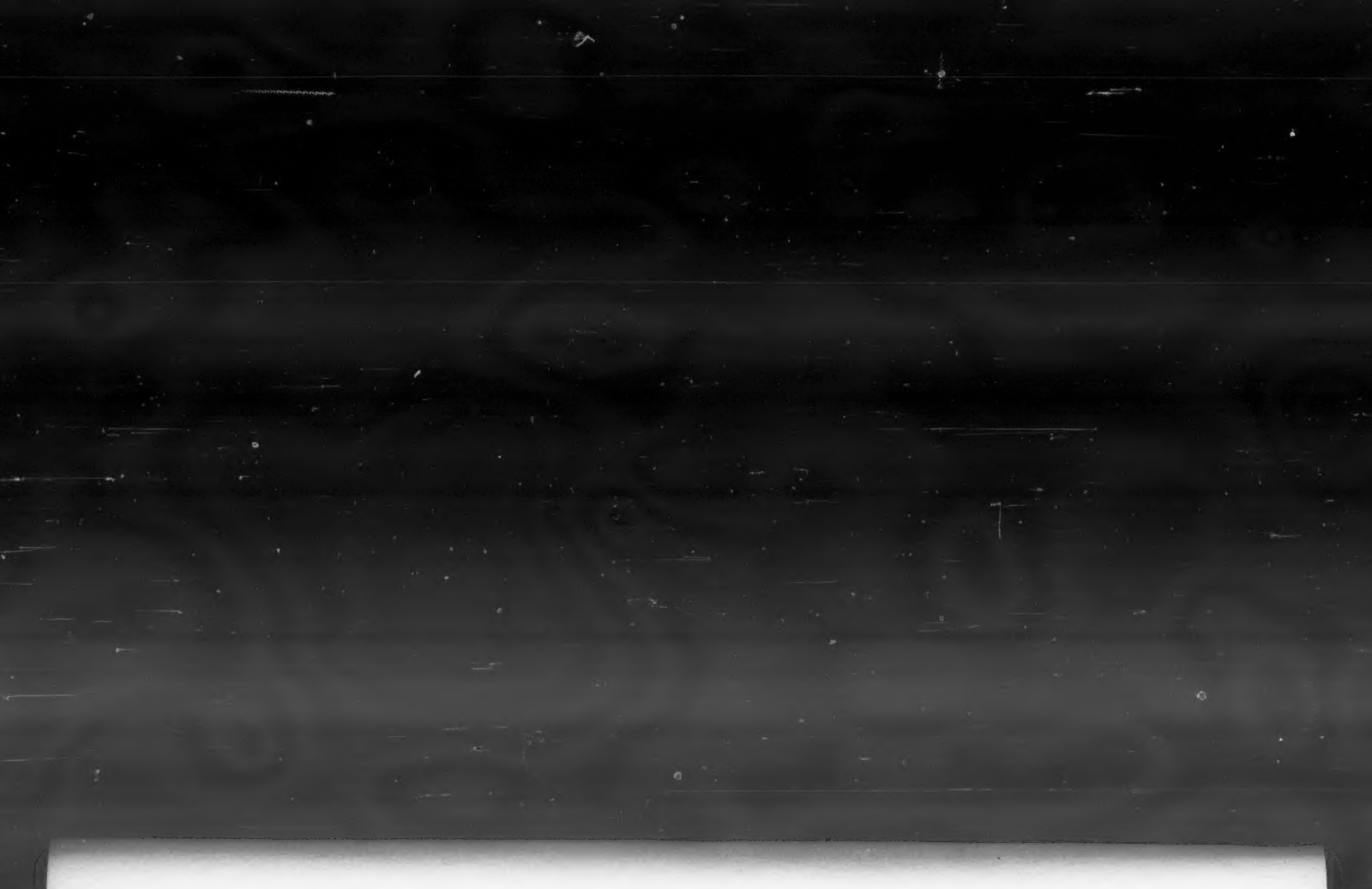
DESCRIPTION OF PLATES

PLATE 180

FIG. 1. Lymphoid hyperplasia in cervical node. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) × 500.

FIG. 2. Lymphocytic infiltration in liver. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) × 500.



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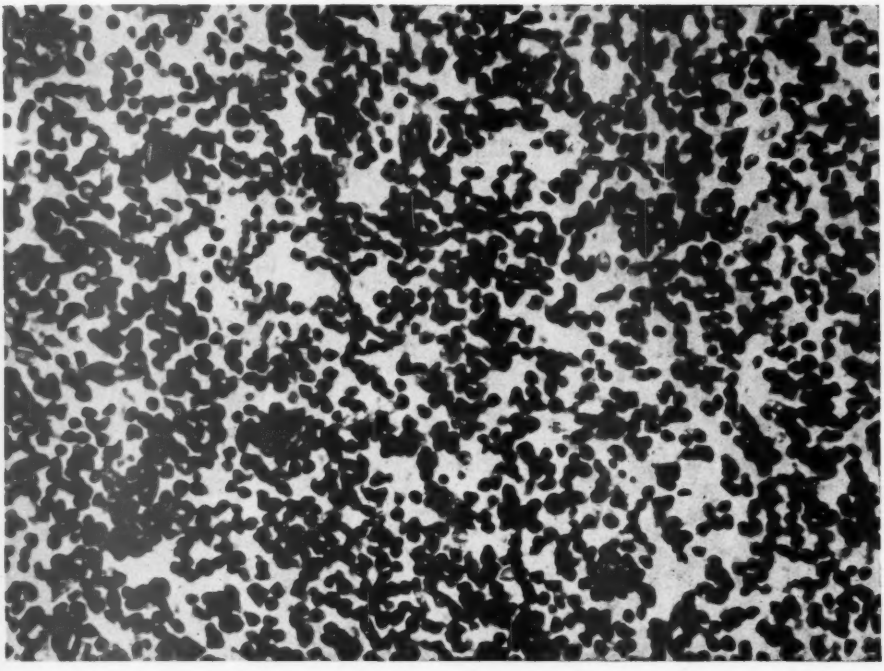

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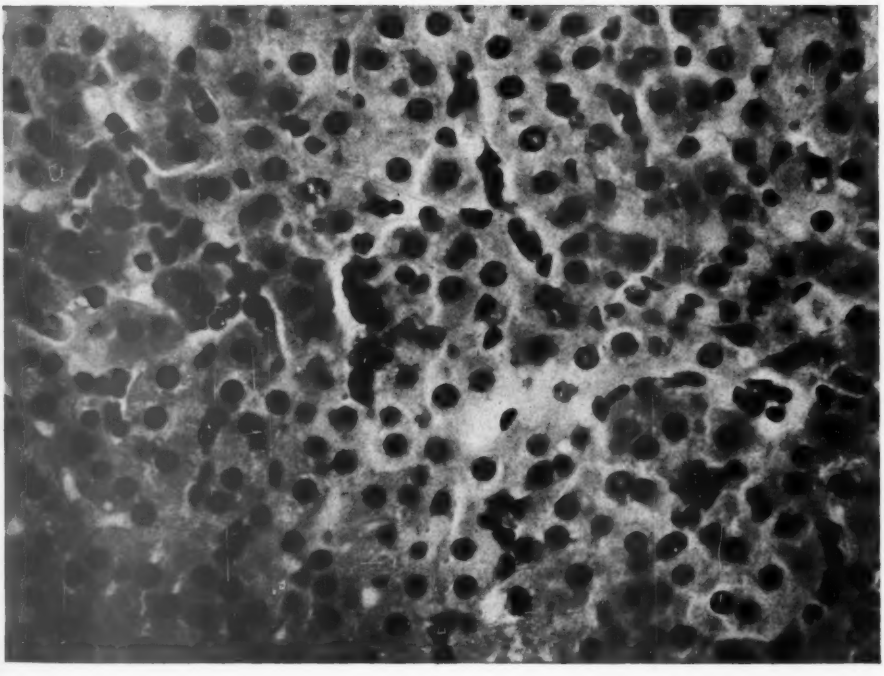

PLATE 181

FIG. 3. Perivascular lymphocytic infiltration in lung. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) × 100.

FIG. 4. Lymphocytic hyperplasia in spleen. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) × 500.

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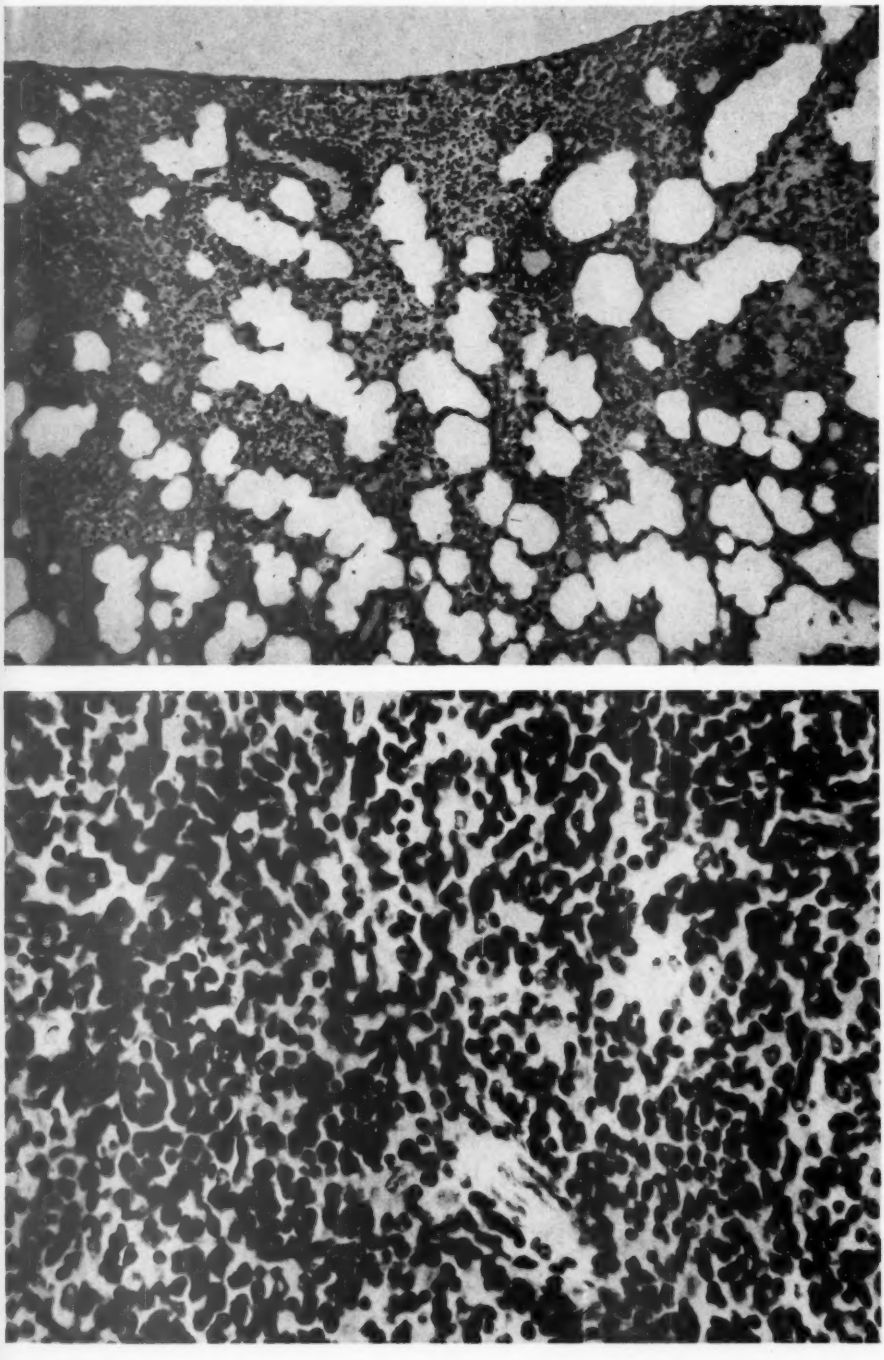
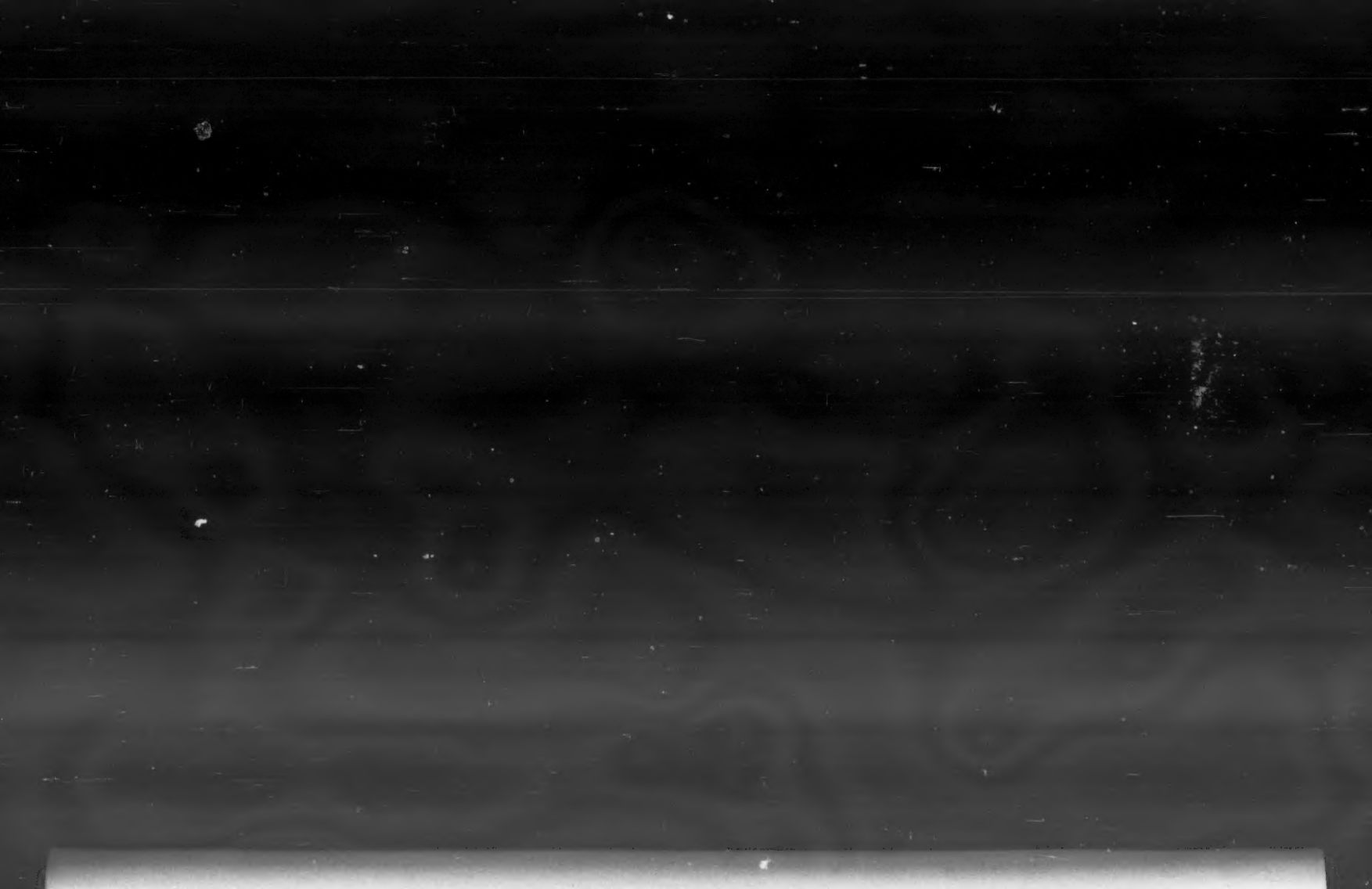


Sawitsky and Meyer Hematopoietic Substances Derived from Urine

PLATE 182

FIG. 5. Normal bone marrow. × 500.

FIG. 6. Lymphocytic infiltration in bone marrow. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) × 500.



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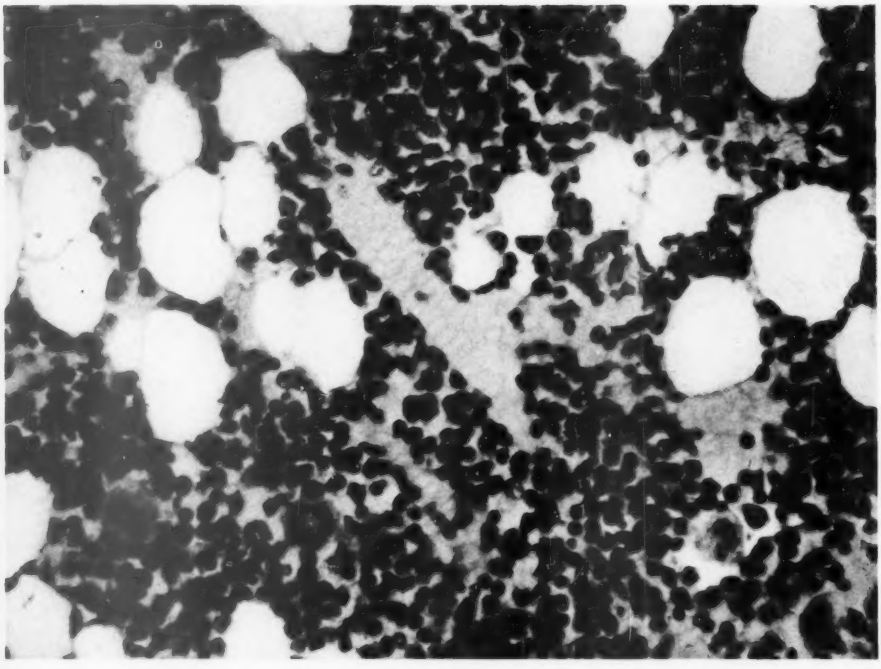

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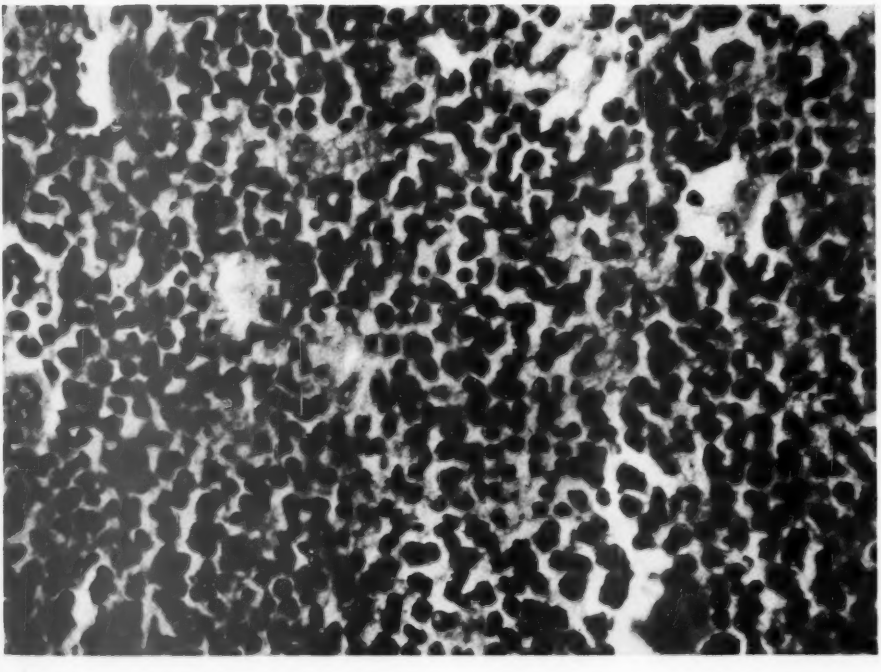
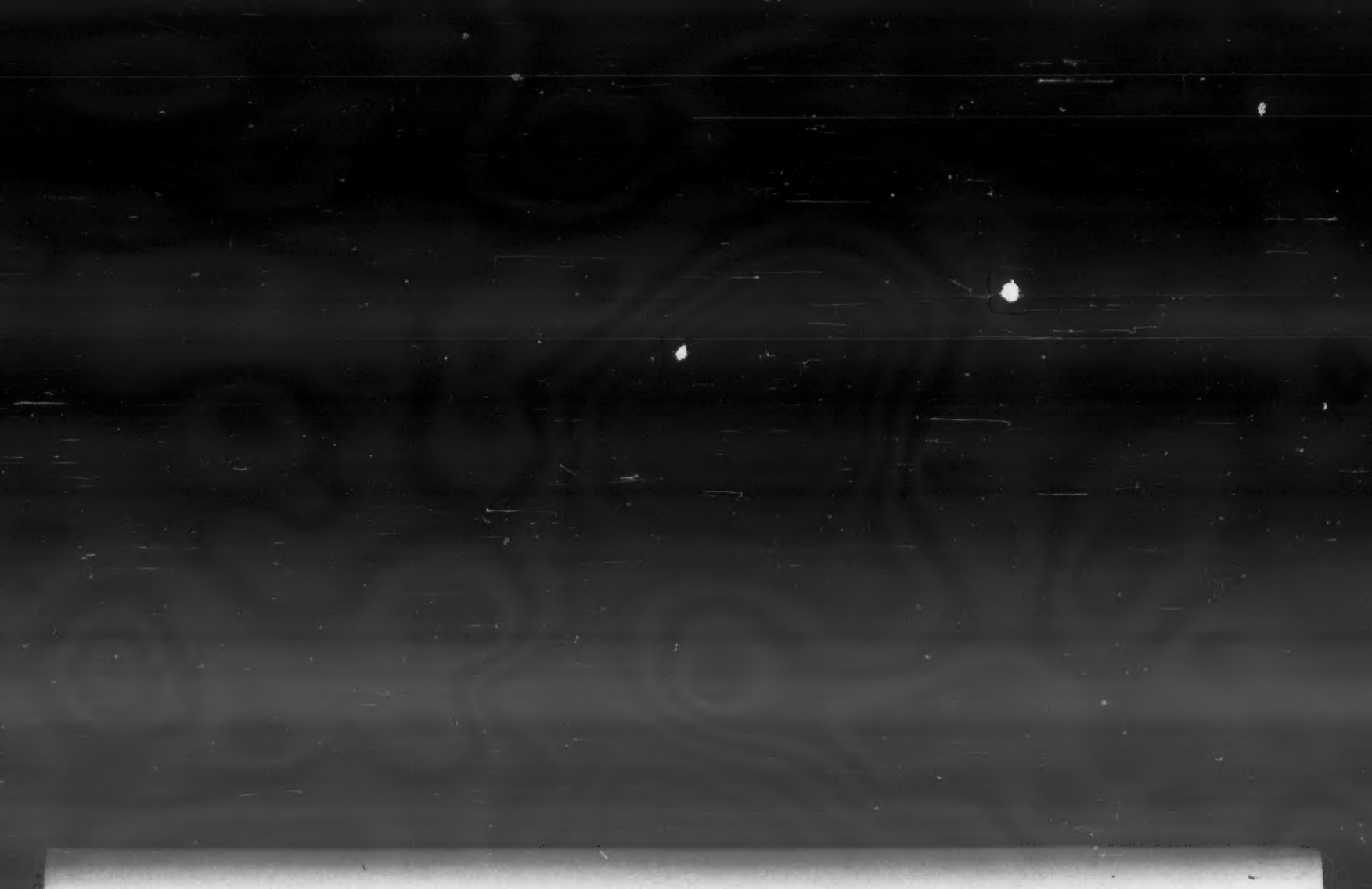
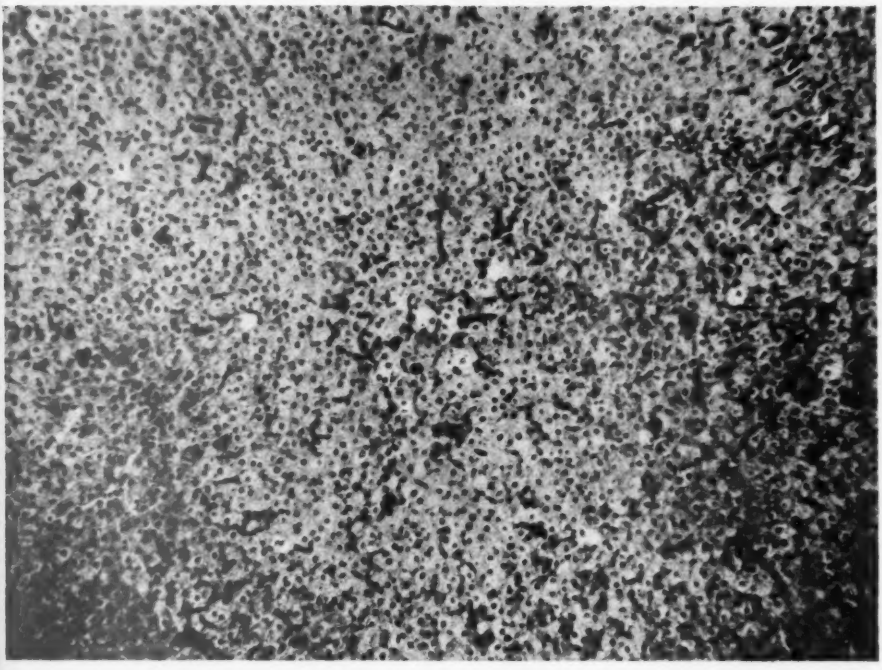


PLATE 183

FIG. 7. Myeloid infiltration in liver. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) × 100.

FIG. 8. Myeloid infiltration in liver. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) × 500.





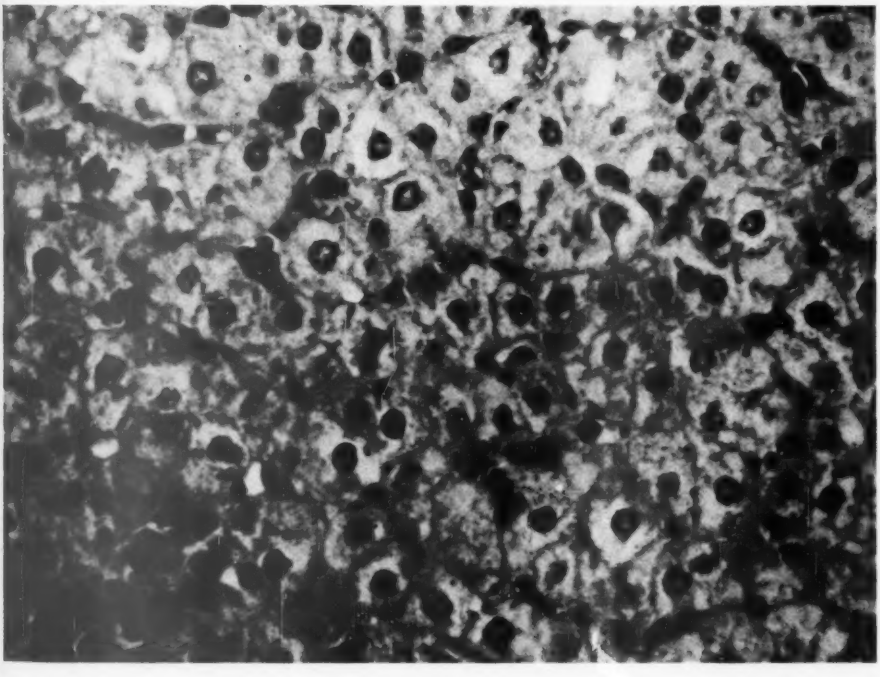


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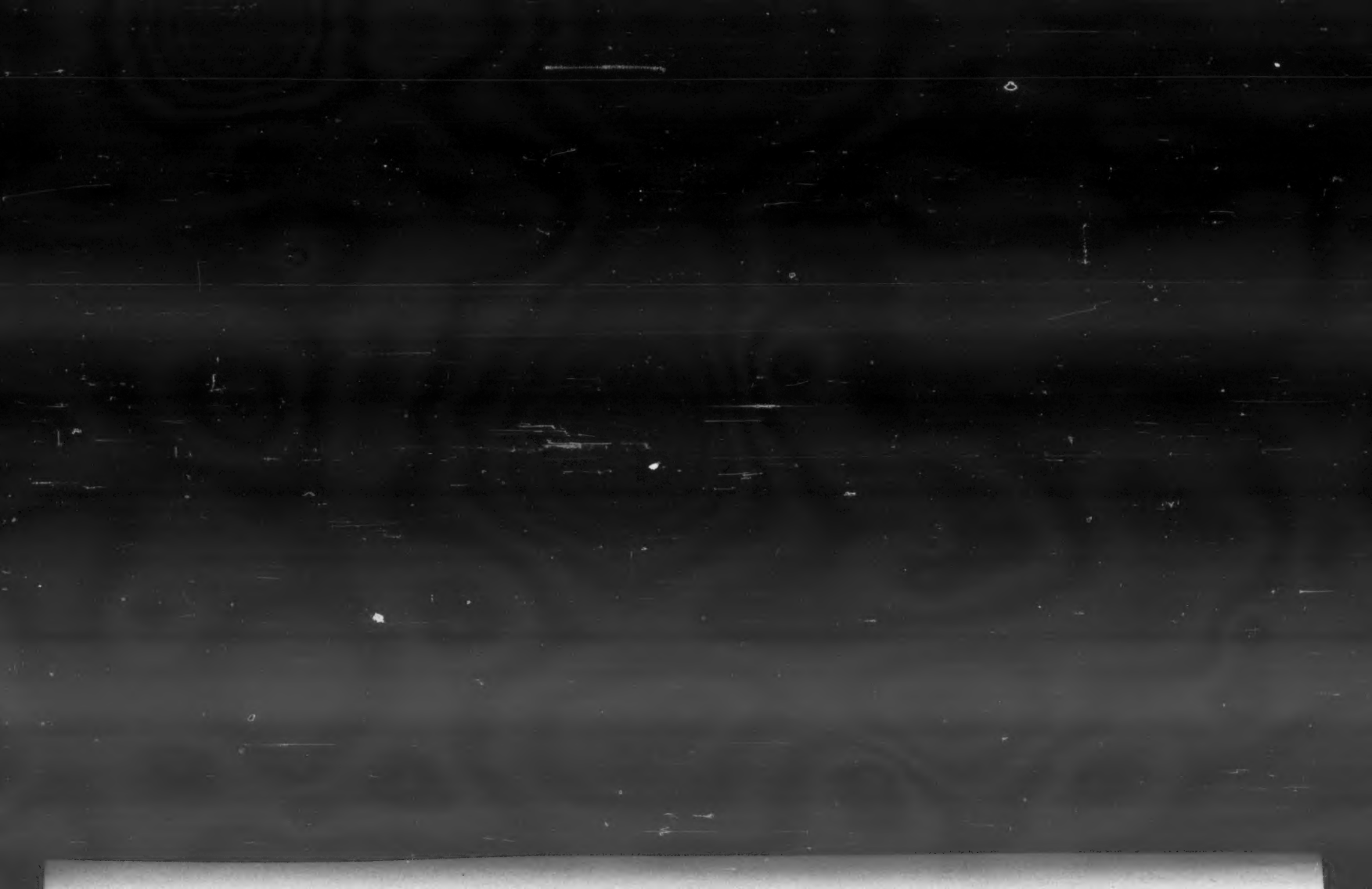
FIG. 9. Plug of myeloid cells in pulmonary arteriole. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) × 500.

FIG. 10. Myeloid hyperplasia in bone marrow. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) × 500.



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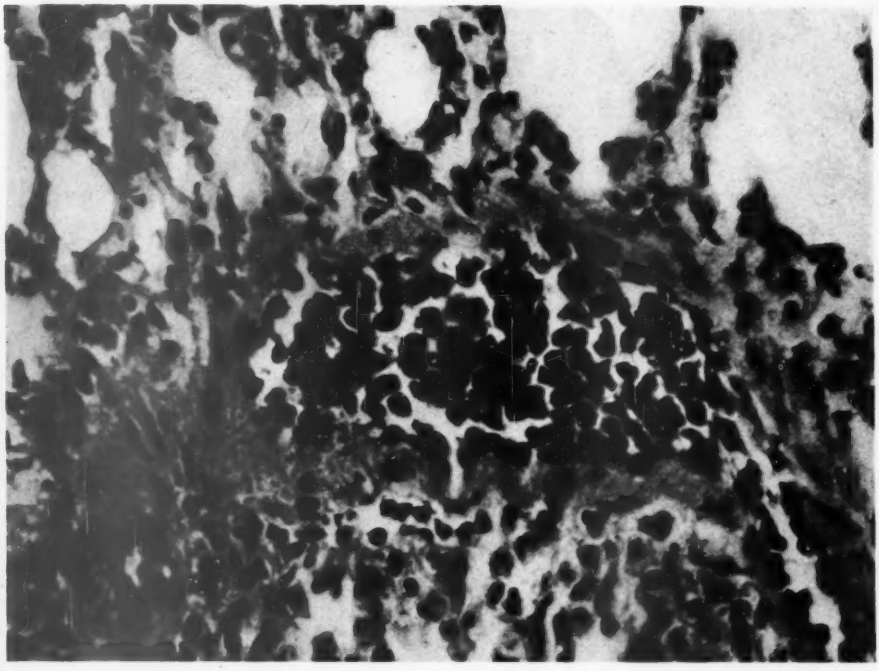

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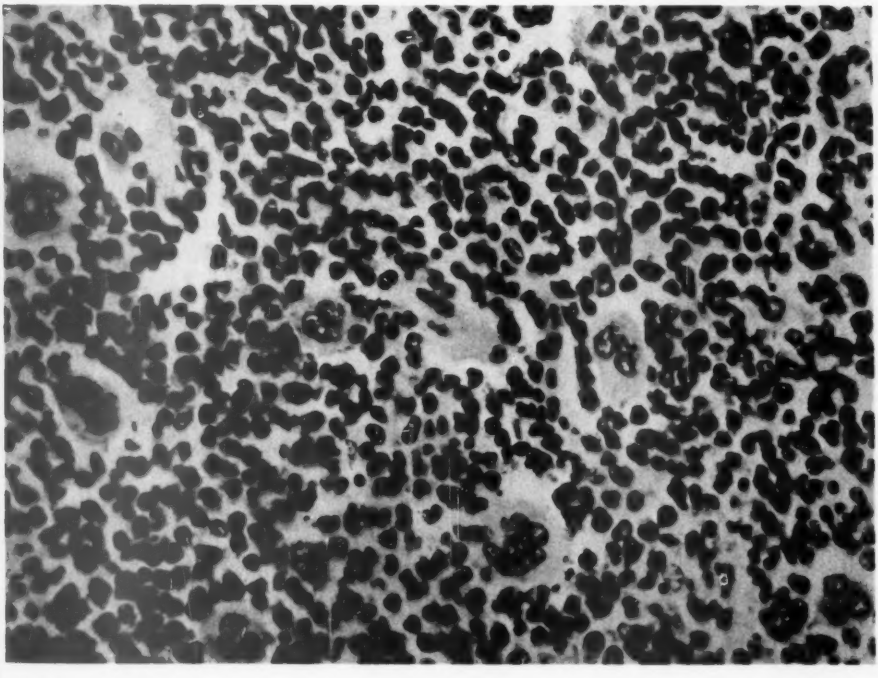

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